Acta Pharmaceutica Sinica B 2023;13(8):3300–3320

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

Plant-derived nanovesicles: Further exploration of biomedical function and application potential

Aixue Li[a,b,c,†], Dan Li[b,c,†], Yongwei Gu[b,c,†], Rongmei Liu[a,b,c], Xiaomeng Tang[b,c], Yunan Zhao[b,c], Fu Qi[a,b,c], Jifu Wei[d,e,f,*], Jiyong Liu[a,b,c,g,*]

[a]*College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250355, China*
[b]*Department of Pharmacy, Fudan University Shanghai Cancer Center, Shanghai 200032, China*
[c]*Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China*
[d]*Department of Pharmacy, Jiangsu Cancer Hospital, Nanjing 210009, China*
[e]*Jiangsu Institute of Cancer Research, Nanjing 210009, China*
[f]*The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing 210009, China*
[g]*Department of Pharmacy, Shanghai Proton and Heavy Ion Center, Shanghai 201315, China*

Received 20 September 2022; received in revised form 21 November 2022; accepted 15 December 2022

KEY WORDS

Plant-derived nanovesicles;
Extracellular vesicles;
Therapeutic agents;
Drug delivery systems;
Clinical application

Abstract Extracellular vesicles (EVs) are phospholipid bilayer vesicles actively secreted by cells, that contain a variety of functional nucleic acids, proteins, and lipids, and are important mediums of intercellular communication. Based on their natural properties, EVs can not only retain the pharmacological effects of their source cells but also serve as natural delivery carriers. Among them, plant-derived nanovesicles (PNVs) are characterized as natural disease therapeutics with many advantages such as simplicity, safety, eco-friendliness, low cost, and low toxicity due to their abundant resources, large yield, and low risk of immunogenicity *in vivo*. This review systematically introduces the biogenesis, isolation methods, physical characterization, and components of PNVs, and describes their administration and cellular uptake as therapeutic agents. We highlight the therapeutic potential of PNVs as therapeutic agents and drug delivery carriers, including anti-inflammatory, anticancer, wound healing, regeneration, and antiaging properties as well as their potential use in the treatment of liver disease and COVID-19. Finally, the toxicity and immunogenicity, the current clinical application, and the possible challenges in the future development of PNVs were analyzed. We expect the functions of PNVs to be further explored to promote clinical translation, thereby facilitating the development of a new framework for the treatment of human diseases.

*Corresponding authors.
E-mail addresses: weijifu@njmu.edu.cn (Jifu Wei), liujiyong@fudan.edu.cn (Jiyong Liu).
[†]These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2022.12.022

1. Introduction

Extracellular vesicles (EVs) are phospholipid bilayer vesicles secreted by cells, that encapsulate a variety of functional nucleic acids, proteins, and other components, and have similar physiological functions to their source cells; they are important mediators of intercellular communication, mediating information transfer and material exchange in prokaryotes and higher eukaryotes[1,2]. EVs have been extensively studied in different scientific fields, such as drug delivery systems (DDSs), disease treatment, and clinical diagnosis, and have been applied in the treatment of cancer, cardiovascular diseases, neurodegenerative diseases, and tissue repair[3,4]. EVs are physiologically active and can avoid phagocytosis of the reticuloendothelial system, penetrate biomembrane barriers, and prolong the half-life of drugs[5,6]. However, the clinical potential of mammalian cell-derived extracellular vesicles (MEVs) is plagued by the complex extraction process, low yield, and high cost. Therefore, the search for easily available and high-yield sources of EVs has become a hot topic for researchers in recent years.

Plant-derived vesicles are abundant in resources, with low production costs and high yield. Regarding safety, plant-derived vesicles do not harbour any zoonotic or human pathogens and have lower immune risks *in vivo*[7]. They are natural disease therapeutics that combine many advantages such as simplicity, safety, eco-friendliness, low cost, and low toxicity[8]. In 2009, when exosome-like vesicles of 50−200 nm containing lectins involved in inflammation, cancer, and autoimmunity were extracted from sunflower by differential centrifugation, the research on plant-derived vesicles in the field of biomedicine officially entered a stage of rapid development[9]. The research results can confirm the vigorous development of plant-derived vesicles: (1) The same kind of plant-derived vesicles have been discovered and used in many fields. For example, ginger-derived vesicles can regulate proinflammatory cytokines to improve the symptoms of colitis in mice, inhibit the activation of microglia to treat encephalitis in hyperlipidaemic mice, and release phosphoric acid by reacting with proteins to treat chronic periodontitis[10−12]; (2) More unexpected species of plant-derived vesicles are being developed for disease treatment, such as those from tea flowers[13], aloes[14], corn[15], beetroots[16], shiitake mushrooms[17], blueberries[18], etc.

As the storage of DNA, RNA, lipids, proteins, and other molecules, plant-derived vesicles are closely involved in plant growth and development, defence response, cargo delivery, and plant-microbe symbiosis, which means that they effectively protect the carried components and direct them to the target site[19,20]. Naturally, the research on plant-derived vesicles mainly focuses on the study of plant physiological and pathological processes and their application in the biomedical field. These two directions complement each other and jointly promote our deeper understanding of plant-derived vesicles, and this article focuses on the latter. Most of the plant-derived vesicles currently used in the biomedical field are extracted by destroying plant tissue, which is a mixture of artificially produced vesicles and native EVs. And there is still no establishment of the exact biogenesis pathway of plant-derived vesicles, so there is no clear way to distinguish artificially produced vesicles from plant EVs. Therefore, it is not entirely appropriate to refer to them collectively as plant EVs. Specifically, we focus on the therapeutic function of plant-derived vesicles, which are not specific secretory pathways, extraction methods, and sizes, so we collectively call them "plant-derived nanovesicles" (PNVs)[21]. In this review, the biogenesis, isolation methods, physical characterization, components, drug administration, and cell uptake of PNVs are analyzed in detail. Emphasis is placed on elucidating the potential of PNVs as disease therapeutics and promising DDSs through recent studies. Finally, we put forward our unique views on the toxicity and immunogenicity, clinical application, opportunities, and challenges in the future development of PNVs.

2. Biogenesis of PNVs

According to their intracellular origin, biological function, and biophysical properties, MEVs can be divided into exosomes from the endosomal pathway (50−150 nm), microvesicles produced through the outward budding of the cell membrane (150−1000 nm), and apoptotic bodies generated by apoptotic processes triggered by programmed cell death (1−1.5 μm)[3,22]. The existence of cell walls suggests that plant cells have more complex modes of communication than MEVs, which means that it is difficult to explore the mechanism of PNV formation. Sources of PNVs pieced together by existing studies include multivesicular bodies (MVBs), exocyst-positive organelles (EXPOs), vacuoles, and autophagosomes[19].

MVBs are late endosomes encapsulating intraluminal vesicles (ILVs), which are regulated by the endosomal sorting complex required for transport (ESCRT)[23]. The MVB pathway of plant cells is similar to the exosome production pathway of mammalian cells. When plant tissue is infected by barley powdery mildew fungus (*Blumeria graminis* f. sp. hordei, Bgh), MVBs release exosome-like vesicles by fusing with the plasma membrane (PM) or secreting them to specific infection sites[24]. Studies have shown that the MVBs released by plants at the site of fungal infection are marked with ARA6 (a plant unique Rab5 GTPase) and Tetraspanin-8 (TET-8)[25,26]. The former is located at the infection site and remains on the PM for the next round of transport, while the latter is located on the released vesicles, which is similar to the mammalian cell-derived exosome marker CD63 and is expected to be a plant exosome marker. In addition, plant-microbe symbiosis also promotes the fusion of MBVs with the host-derived periarbuscular membrane (PAM), which is continuous with the PM and releases the ILVs to the periarbuscular space between plants and fungi. Another class of PNVs derived from a new organelle, EXPOs, which was discovered by an Arabidopsis homologue expressing exocyst protein Exo70E2 in suspension cells of Arabidopsis and tobacco[27]. EXPOs are morphologically distinct from MVBs and independent of endosomes and autophagosomes, but the Exo70E2-GFP signal does not colocalize with any known

organelle markers; therefore, its biological origin has not been demonstrated. After fusion with the PM, EXPOs release vesicles into the extracellular space, which are 200−500 nm larger than exosomes[28]. Recently, the colocalization of glycosyltransferases with EXPO markers in tobacco suggested that EXPO-derived vesicles may be involved in the secretion of arabinogalactan proteins in plants. In addition, studies have shown that ILVs also exist in vacuoles and contain sRNA and defense-related proteins (Fig. 1); autophagosomes involved in unconventional protein secretion (UPS) in yeast are secreted out of cells[29].

3. Isolation and stability of PNVs

3.1. *Isolation of PNVs*

Isolation refers to the isolation of PNV and non-PNV components and different types of PNVs from each other through different techniques[30]. The International Society for Extracellular Vesicles (ISEV) has classified EV isolation methods according to recovery and specificity[31]. An appropriate isolation method should be selected according to the key experimental issues and the final use of the vesicles, among which basic research and clinical application are the bases for the selection of methods. When identifying specific markers or functions of specific vesicles, highly specific isolation methods are needed, and when certain biomarkers and functions do not directly correlate with the enrichment of vesicles, recovery should be a priority[31−33]. Currently, there is no perfect method that takes into account both recovery and specificity. The existing PNV isolation methods are summarized as follows (Fig. 2).

3.1.1. *Pretreatment before PNVs isolation*

Plants come from a wide variety of sources, and PNVs can also be extracted from seeds, roots, stems, fruits, leaves, and other parts of different plants; therefore, specific pretreatment of different parts of different plants is required before the isolation operation. Juicy fruits (such as grapefruit[34] and lemon[35]) can be directly squeezed after cleaning to obtain initial samples of PNVs. Plant roots and stems (such as ginseng[36] and ginger[37]) are hard and have little juice, and thus samples need to be obtained by a combined grinder and juicer; an appropriate amount of PBS should be added in the process to promote the production of juice. Plant leaves and seeds (such as *Arabidopsis thaliana* leaves[38] and sunflower seeds[9]) need to undergo a vacuum infiltration-centrifugation procedure in an infiltration buffer to obtain apoplastic fluids before subsequent

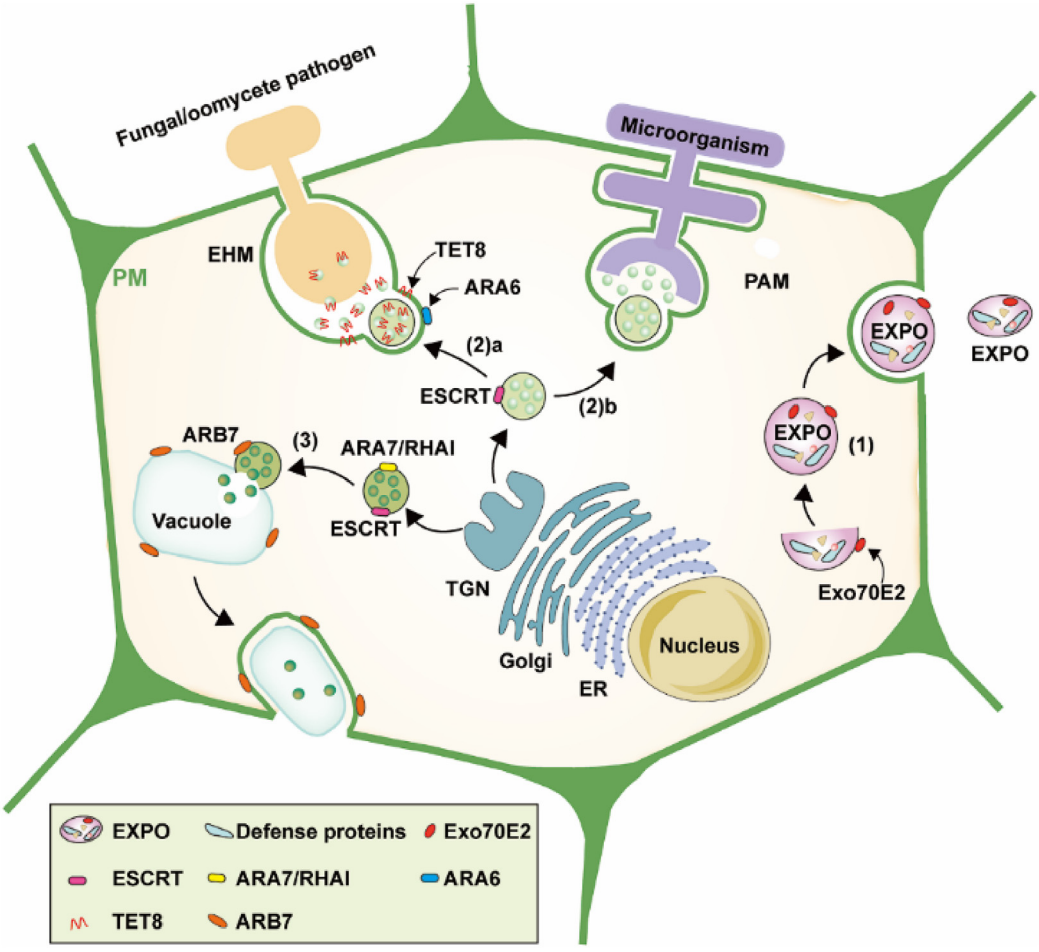

Figure 1 Biogenesis of plant-derived nanovesicles. (1) Secretion of EXPO; (2)a MVBs are secreted to designated sites of infection; (2)b MBVs fuse with host-derived PAM; (3) Vacuole fuses with PM to release ILV. EHM, extrahaustorial membrane; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; EXPO, exocyst-positive organelle; PAM, peri-arbuscular membrane; PM, plasma membrane; TGN, trans-Golgi network.

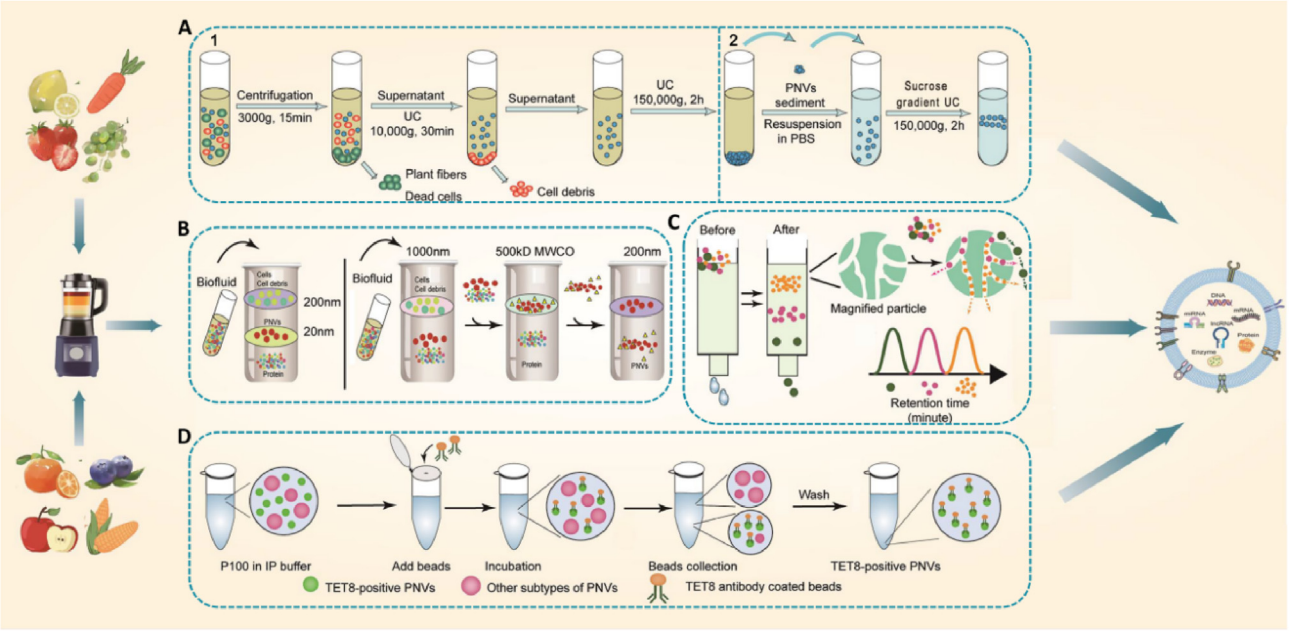

Figure 2 Isolation and preparation of plant-derived nanovesicles. Plant-derived nanovesicles were isolated and purified by (A1) differential ultracentrifugation, (A2) sucrose gradient centrifugation, (B) ultrafiltration, (C) size exclusion chromatography, and (D) immunoaffinity capture.

operations. For the specific operation process, please refer to previous studies[39,40].

3.1.2. *Differential ultracentrifugation*

According to a global survey in 2015, differential ultracentrifugation is the most commonly used EV isolation method, which has high repeatability and minimal impact on samples[41]. The number, size, and purity of vesicles vary greatly among plant species, so the choice of centrifugation speed cannot be limited to a certain set of protocols, but needs to be tailored for specific plants. Routine steps: (1) 500−3000 $\times g$ low-speed centrifugation for 10−15 min to remove large particles such as plant fibers; (2) 2000−10,000 $\times g$ medium-speed centrifugation for 20−40 min to remove cell debris; and (3) 100,000−150,000 $\times g$ high-speed centrifugation for 1.5−2 h to obtain PNVs (Fig. 2A1). The speed and time of the centrifugation are gradually increased, and 0.22- or 0.45-micron pore size filters can be used in the middle of the centrifugation step to ensure the purity of the PNVs. In addition, in order to prevent damage to the morphology and structure of PNVs by forceful centrifugation, it is necessary to lay a thin layer of sucrose cushion at the bottom of the centrifuge tube[42].

It should be noted that the ultrahigh speed and ultralong time of ultracentrifugation may lead to the coprecipitation of protein or protein/RNA aggregates; therefore, density gradient centrifugation with sucrose or iodixanol is usually combined after differential ultracentrifugation (Fig. 2A2)[43,44]. While purifying PNVs, this method can also separate PNVs with different particle sizes in a solution interlayer of a specific density. Usually, the sample solution to be separated is added to the centrifuge tube, and the needle then is inserted into the bottom of the tube to add the gradient solution (8%, 30%, 45%, 60%) in order from low to high concentration. Ultracentrifugation is then performed[8,45]. However, experiments involving ultracentrifugation require high-level laboratories and professional operators, which are expensive and difficult to popularize in all regions[46].

3.1.3. *Size-based isolation*

Ultrafiltration is a typical PNV isolation method based on size and molecular weight, which takes the pressure difference between the two sides of the membrane as the driving force and the ultrafiltration membrane as the filter medium to purify, separate, and concentrate the original solution under a certain pressure (Fig. 2B)[47−49]. Researchers used a 100K filter to extract high-purity, near-circular PNVs from the leaves and stems of *Dendropanax morbifera*[50]. This method is simple to perform and eliminates the limitations of ultracentrifugation using large equipment. However, it should be noted that the samples with more PNVs and miscellaneous proteins easily block the filter membrane and affect the recycling of the filter, so the method is more suitable for thinner samples with more juice.

Size exclusion chromatography (SEC) is a technique for separating molecules or particles based on their hydrodynamic volume (Fig. 2C)[51,52]. The researchers compared the ability of ultracentrifugation, polyethylene glycol precipitation, and ultrafiltration combined with SEC to extract PNVs from cabbage. In terms of purity, SEC yielded results that were more than 20 times higher than those derived from the other two methods. SEC is considered the most standard method for the isolation of PNVs, which guarantees the highest purity without loss of yield, and is now used for the extraction of PNVs from tomatoes, cucumbers, and peppers[53,54].

3.1.4. *Immunoaffinity capture*

Immunoaffinity capture is the most advanced method for the isolation of specific types of PNVs (Fig. 2D)[55]. This method uses antibodies to capture PNVs with specific proteins on the surface, which can significantly improve the enrichment of vesicles carrying specific proteins and achieve precise isolation of PNVs[56]. Recently, He et al.[40] coated magnetic beads with antibodies targeting the PNV marker TET8, which specifically recognizes the EC2 domain of TET8, and successfully extracted TET8-positive PNVs from samples after ultracentrifugation.

3.1.5. Other isolation methods

Polyethylene glycol precipitation is often used to extract exosomes from mammalian cells[57,58]. Now, researchers use it to extract ginger-derived PNVs (GgPNVs). The results show that the biological characteristics and material composition of GgPNVs are similar to those of ultracentrifugation, and the yield of PNVs extracted by PEG6000 is higher than that of PNVs extracted by PEG8000 and PEG4000[59]. Recently, the research team also used an exosome isolation kit to extract vesicles from ginger and cherry, usually combined with low-speed differential centrifugation, and the sample was centrifuged through a 0.45-μm micromembrane before adding precipitation reagent[60,61]. In addition, Savcı et al.[62] used an aqueous two-phase system containing PEG/dextran to separate grapefruit-derived PNVs (GfPNVs). The characteristics of the commonly used methods for isolating PNVs are shown in Table 1[54,63−68].

3.1.6. Combined isolation method

There are more macromolecules (such as cellulose and starch) in plant extracts, and the components are more complex, which renders the extraction of vesicles more difficult[69,70]. The combination of different isolation methods may extract nanovesicles with higher purity than a single method, and therefore the combined isolation method has been deeply explored and applied in recent years. The most common is the combination of differential ultracentrifugation and ultrafiltration, which has a wide range of applications, low cost and power consumption, and a high degree of product standardization[71]. Fikrettin Şahin's team[72] combined an exosome isolation kit with a size-based isolation method. First, the PNVs were crudely extracted using the exosome purification kit, and the extract was then resuspended and passed through a 0.22-μm filter membrane. The particle size of the vesicles isolated by this method was between 40 and 100 nm, and the shape was uniformly spherical. Yang et al.[73] combined electrophoresis and dialysis, put the extracted supernatant into a 300 kDa dialysis bag, and then placed it into a 300 mA current. After 30 min, the direction of the current was changed to successfully separate lemon-derived PNVs (LPNVs) and bitter melon-derived PNVs (BmPNVs).

3.2. Stability of PNVs

The development of an appropriate PNV extraction method is the first step to the success of the experiment, and how to choose the matrix used for EV extraction and how to store are the keys to determining the stability of PNVs[74]. The activity and size of PNVs, *i.e.*, biological stability and physical stability, are the criteria for measuring stability, while storage temperature and living environment are important factors affecting the stability of PNVs[75]. In 2013, the ISEV suggested storing samples at −80 °C and using PBS as the matrix. However, in 2018, it no longer promoted specific methods but recommended that specific schemes should be described more accurately by considering the nature of different types of vesicles[31]. Indeed, there is no consensus on the impact of storage on EVs function and properties. Recently, Gelibter et al.[76] evaluated the effects of −80 °C storage, cryoprotective agents, and freeze−thaw cycles on vesicles, showing that −80 °C freezing resulted in a time-dependent increase in vesicle size (there was no difference between the four-week storage vesicles and fresh vesicles, but there was a significant change over six months). GfPNVs remained stable for more than one month even at 4 °C storage[77]. Second, the addition of cryoprotectants and the freezing and thawing rates had little effect on the properties of EVs, while the number of EVs decreased and the particle size increased when the number of freeze−thaw cycles exceeded one[76]. Our unpublished research also found that the particle size of ginseng-derived PNVs (GsPNVs) increased slightly and the vesicle fragments increased during the second freezing and thawing.

The stability of EVs in their environment is the key to their functioning, and PNVs have the advantage of being stable in different conditions. Edible tea flower-derived PNVs showed no

Table 1 Advantages and disadvantages of main isolation methods for plant-derived nanovesicles.

Method	Equipment	Advantage	Disadvantage	Ref.
Differential ultracentrifugation	Ultracentrifuge	Low cost; large sample capacity; high sample capacity	Time-consuming; equipment dependence; complexity	63
Density gradient	Ultracentrifuge	High purity; high separation efficiency; less affected by miscellaneous components	Time-consuming; equipment dependence; complex process; loss of sample; low yield	64
Ultrafiltration	Ultrafiltration membrane	Fast procedure; no limitations on sample volume; pure preparations; low cost	Vesicles trapping; loss of PNVs; possibility of clogging	65
Size exclusion chromatography	Chromatographic column	Purity; reproducibility; preserves vesicle integrity; scalability; prevents PNVs aggregation	Complexity; limitations on sample volume; specialized equipment; a small number of samples; high cost	54
Immunoaffinity capture	Magnetic bead, antibody	Purity and high selectivity; convenient operation; low cost	High reagent cost; low yields; difficult to analyze complete vesicles	66
Polyethylene glycol precipitation	Polyethylene glycol	The simplicity of procedure; low cost; preservation of PNVs integrity	Retention and contamination of the polymer	67
Exosome isolation kit	Exosome isolation kit	Suitable for small samples; simple steps; fast procedure	Impurity; low production; expensive reagents	68

PNVs, plant-derived nanovesicles.

change in zeta potential or particle size in simulated fluid in the stomach, small intestine, and colon and remained stable in the gastrointestinal tract and blood after oral or intravenous injection[13]. In contrast, the particle size of GgPNVs increased from 177.9 to 348.5 nm in the stomach-like solution (pH ~ 2.0) and further to 507.7 nm in the small intestine-like solution, and the zeta potential of PNVs changed from negative to positive in stomach-like solution and reversed to negative in small intestine-like solution[10]. Although different pH can change the size and zeta potential of PNVs, PNVs are still nanoscale in size, with good stability, and the potential changes conform to their natural properties. In addition, the stability of PNVs in the gastrointestinal tract suggests that PNVs may be high-quality carriers of nutrients in fruits and vegetables, which confirms that PNVs may have unexpected advantages as oral DDS. In addition to the results of particle size and potential, we believe that the evaluation of the composition, activity, and function of PNVs is also indispensable because the comprehensive evaluation of stability is one of the key contents of the clinical transformation of PNVs.

4. Characterization of PNVs

4.1. Physical characterization

The quantification and characterization of PNVs after isolation aid in determining whether they can be used for disease treatment. The particle size range of PNVs is larger than that of MEVs and varies greatly due to different plant species; however, the size distribution of PNVs whether natural or reconstituted by extrusion and other methods is usually 30−500 nm[78]. The sizes of kiwifruit, lemon, grapefruit, and blood orange PNVs obtained by differential ultracentrifugation were detected by nanoparticle tracking analysis (NTA). Although the number of PNVs from conventional agriculture and organic agriculture differed, the average particle size ranged from 140 to 220 nm[79]. Dynamic light scattering (DLS) is also a commonly used technique to determine the size distribution curve of small particles in suspensions. The researchers combined DLS and NTA to determine the size distribution of PNVs derived from the stems and leaves of bitter gourd while determining the concentration of PNVs. The numbers of PNVs extracted from stems and leaves (4.98×10^8/g and 1.53×10^9/g, respectively) were different, but the particle sizes were similar, both in the range of 30−200 nm[50]. It is worth mentioning that PNVs have a wide range of particle size, so the method of obtaining uniform particle size by recombination methods such as extrusion have been developed rapidly.

The zeta potential of PNVs generally ranges from −100 to 0 mV, indicating that PNVs can exist independently of each other without aggregation[80]. The average zeta potential of 190 nm PNVs from *Asparagus cochinchinensis* (Lour.) Merr. is −21 mV, the average potential of broccoli-derived PNVs (BPNVs) is −17.1 mV, and the average potential of aloe vera-derived PNVs (AvPNVs) is only −7.4 mV[14,81,82]. Even among the PNVs of the same family, there are obvious differences, the average zeta potential of turmeric-derived PNVs is −21.7 mV, while the zeta potential of GgPNVs is −26.6 mV[10,60]. However, the zeta potentials of PNVs of the same genus were similar. The average zeta potentials of Rabex- and Cabex-derived PNVs are −15.2 and −14.8 mV, respectively[53].

Most of the PNVs obtained by direct extraction were similar in shape to cells and had a spherical lipid bilayer structure, a feature shared by MEVs and PNVs. The structure of the reconstituted PNVs after lipid extraction was similar to that after natural extraction, but the GfPNVs exhibited a round multilayer flower-like structure[77]. The size and structural advantages of PNVs suggest their potential as therapeutic agents or drug delivery vehicles to treat diseases by intravenous injection and oral administration.

4.2. Identification of components

PNVs contain components such as lipids, proteins, and nucleic acids. Due to their complexity and heterogeneity, there is currently no database of PNV components[83]. However, identifying the natural compounds contained in PNVs is essential to explain the mechanisms underlying their multifaceted therapeutic effects.

4.2.1. Lipids

Lipids are one of the most important components of EVs, but PNVs contain different lipid components from MEVs. The lipids contained in MEVs are mainly phosphatidylcholine (PC) and cholesterol, but the specific ratio is related to the source cell. The lipid composition of different PNVs varies[84−86]. The lipidomics of GsPNVs showed that they were rich in digalactosyl monoacylglycerol (59.4%), phosphatidyl ethanolamine (6.8%), and ceramide (Cer, 13.8%), while aloe gel-derived PNVs had the highest content of glucose ceramide (43.55%), followed by Cer (14.98%), phosphatidic acid (18.12%) and PC (3.85%)[14,87]. Moreover, the lipid composition of PNVs is an important contributor to their role as therapeutic agents. PA in GgPNVs binds to HBP35 protein in *Porphyromonas gingivalis*, thereby inhibiting the growth of *P. gingivalis* and effectively treating periodontitis[12]. The Cer of GsPNVs affects macrophage polarization by activating TLR4, ultimately reversing the immunosuppressive tumor microenvironment[87].

4.2.2. Proteins

Protein secretion is necessary for cells to perform normal life activities, and is usually divided into conventional protein secretion (CPS) and UPS. In plant cells, CPS starts from the endoplasmic reticulum, with proteins then passing through the Golgi apparatus and the trans-Golgi network (TGN) and, finally, secreted from the plasma membrane into the extracellular space[88]. UPS usually refers to the secretion of proteins that do not signal peptides or other cytosolic substances, which is also one of the important pathways leading to the formation of PNVs[19,89]. Most of the proteins contained in PNVs are cytoplasmic proteins (actin, protease) and membrane proteins (aquaporin), which can be used as channels and transporters[90]. A large number of proteins exist on the surface of PNVs and mediate intercellular communication, but there is currently no clear specific marker for PNVs. The protein content of PNVs is lower than that of MEVs, both of which have the same type of protein, but the protein composition is quite different. Citrus PNVs contain heat shock proteins commonly found in MEVs, but there is only a 56.7% of protein overlap between the PNV protein dataset and the exosome database ExoCarta[91,92]. Moreover, PNVs proteins (such as aquaporins) can improve the stability of PNVs, so the analysis of the protein composition of PNVs is of great significance for the development of drug delivery carriers based on PNVs[93].

4.2.3. RNAs

PNVs are associated with a variety of RNAs and can deliver these molecules to target sites to affect gene expression. PNVs can transfer sRNAs into fungal cells, and 31 of the 42 transferred host sRNAs have existed in PNVs[94–96]. The transferred host sRNA was not digested by nucleases, indicating that the sRNA is present within the PNVs rather than on the surface[26]. RNA-binding proteins (AGO1, RH11, RH37) and sRNAs are enriched in TET8-positive PNVs, and these RNA-binding proteins contribute to the selective sorting and stabilization of sRNAs within PNVs[40]. MiRNAs are also sRNAs of approximately 22 nt, that exist in various bodily fluids through active secretion or passive leakage through membrane structures, and can bind to mammalian target mRNAs to play a role in cross-border communication[97]. GsPNVs were used as carriers to efficiently transfer 20 kinds of miRNAs into bone marrow mesenchymal stem cells (BMSCs), and regulate 19 target genes in BMSCs, including Tmem100, Vrk1, and LOC103689968, which are related to neural differentiation, maturation, and functionalization[7]. MiR-4057 in honey-derived PNVs can effectively inhibit the activity of the NLRP3 inflammasome[98]. Plant miRNA 3′-ends are naturally modified by 2′-*O*-methylation to protect them from degradation and uridylation, so they are more stable than animal miRNA in most environments, which reveals the potential of plant miRNA as a disease therapeutic agent[99,100].

5. Administration and cellular uptake of PNVs

5.1. Administration of PNVs

The mode of administration is closely related to the type of disease and the nature of the therapeutic carrier[101]. Different modes of administration affect drug distribution[102]. Compared with the control group, GsPNVs were mainly located in the liver and spleen 72 h after intravenous (i.v.) and intraperitoneal (i.p.) administration, and mainly in the stomach and intestine after intragastric (i.g.) administration, but no signal was detected in the heart, lung, kidney, or brain with the three administration methods[87]. Honey-derived PNVs successfully alleviated the inflammatory symptoms of mice with acute liver injury by i.p., but the absorption of bioactive components in the gastrointestinal tract and the anti-inflammatory function of honey-derived PNVs by the oral route need to be further investigated[98]. The oral approach is very popular because of good patient compliance, a wide treatment window, and convenient administration, but its efficacy for most diseases is limited; thus, it is at a disadvantage in nanocarrier-targeted drug delivery research[103,104]. Surprisingly, recent studies have shown that oral administration of edible tea flowers-derived PNVs (TfPNVs) in the high-dose group achieved the same therapeutic effect as i.v. injection on breast cancer lung metastasis model mice, but the Simpson index of the former group was higher than that of the latter, indicating that the oral route increased the abundance and diversity of gastrointestinal microbiota[13]. Coincidentally, GgPNVs have incomparable advantages in the treatment of colitis by oral route compared with the i.v. injection or systemic route, such as the absence of damage to the skin and mucous membranes, fewer adverse reactions, and can be preferentially located in the inflamed colon[10].

5.2. Cellular uptake of PNVs

The interaction between PNVs and receptor cells is the key factor for their effective utilization after administration. It is generally believed that EVs interact with recipient cells through fusion and surface protein interactions that trigger signal transduction in target cells, activation of EV-bound surface proteins, and endocytosis[105]. Endocytosis is the main method by which PNVs play a role with high cell-type specificity. RAW 264.2 and colon 26 uptake GgPNVs *via* caveole-mediated endocytosis with endocytosis efficiencies of 98% and 91%, respectively[10]. HepG2 cells mainly take up *A. cochinchinensis*-derived PNVs through macropinocytosis[81]. The uptake of GfPNVs by A549 cells was inhibited by cytochalasin D, nocodazole, and chlorpromazine, suggesting that tumor cells internalize PNVs through clathrin-mediated endocytosis and macropinocytosis[77]. Cellular uptake of PNVs is also closely related to the protein expression level on the cell surface. After the downregulation of CD48 on the surface of hepatocellular carcinoma cells, the endocytosis of garlic-derived PNVs decreased significantly[106]. In addition, the pathway of cellular internalization of PNVs is most likely a bioactive process, because the uptake efficiency of LPNVs decreases significantly at 4 °C, while GfPNVs were efficiently taken up at 37 °C and slowly taken up at 4 and 20 °C[73,77].

6. PNVs as therapeutic agents

Fresh plant materials can provide the nutrients needed by the human body, including proteins, vitamins, and minerals, and the active substances (such as phenols and polysaccharides) contained in plants are a natural treasure trove for disease treatment[107,108]. PNVs have been shown to have similar pharmacological effects to those of the original plant, and can protect its components, thus achieving effective delivery to the target site (Fig. 3).

6.1. Antitumor

Due to the stubborn drug resistance of tumors and the side effects of drugs, the development of antineoplastic drugs is still a difficult problem faced by scientists[109,110]. In recent years, PNVs have been widely studied due to their safe source, low side effects, and good antitumor efficacy. PNVs derived from *A. cochinchinensis* can suppress tumor proliferation, upregulate the apoptosis-related factor caspase-9 in hepatocellular carcinoma cells, and induce apoptosis. Because of its special tumor-selective inhibition activity, the damage to normal hepatocytes is negligible[81]. Corn-derived PNVs synergistically exert antitumor effects through the indirect effect of activating immune cells such as macrophages to infiltrate tumor cells to produce the proinflammatory cytokine tumor necrosis factor α (TNF-α) and the direct effect of inhibiting colon 26 cell proliferation[15].

Increased intracellular reactive oxygen species (ROS) content not only triggers mitochondrial damage but also arrests the cell cycle, thereby exhibiting anti-proliferative, anti-migratory, and anti-invasive activities against cancer cells. BmPNVs trigger ROS-mediated cancer cell death by increasing the Ca^{2+} concentration while downregulating NLRP3 expression, enhancing cytotoxicity, and reversing the drug resistance caused by oral squamous cell carcinoma. The production of ROS is mediated by MAP30 protein, and the downregulating of NLRP3 is related to 11 microRNAs. The concentration of protein and RNA in bitter melon juice was more than 100-fold higher than that of BmPNVs, but their therapeutic effects were similar in the same volume, indicating the strong antitumor activity of BmPNVs and their great potential as therapeutic agents[111]. The combined action of the rich bioactive

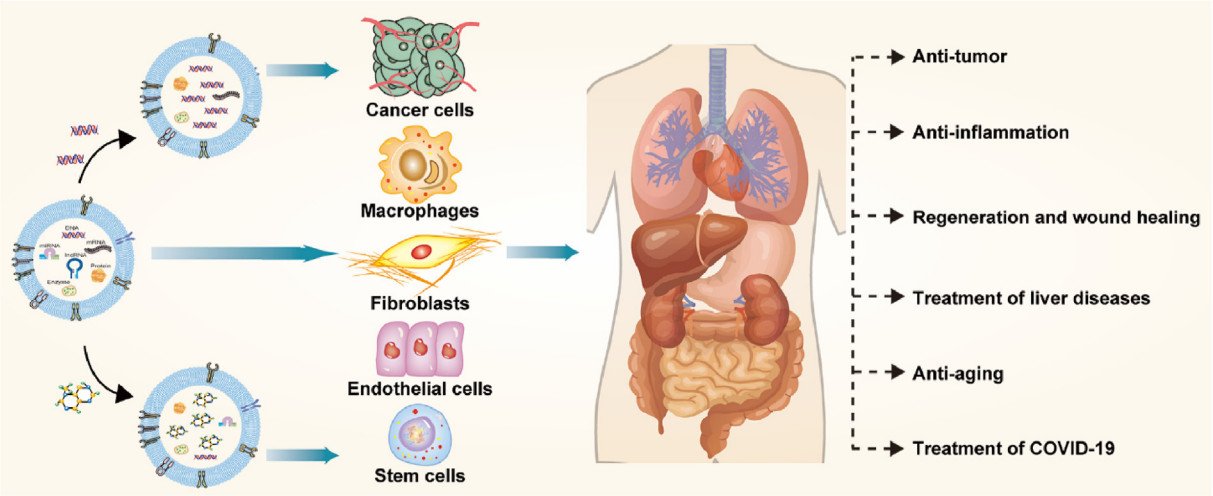

Figure 3 Plant-derived nanovesicles with targeting capabilities as drug delivery carriers for various diseases.

components (proteins, lipids, polyphenols) of tea flower-derived PNVs triggers high oxidative stress in breast cancer cells, leading to mitochondrial damage and cell cycle arrest[13]. Coincidentally, LPNVs mediated S-phase arrest of the cell cycle by upregulating GADD45A in gastric cancer cells, and promoted the production of ROS in gastric cancer cells, leading to apoptosis[73].

Folic acid (FA)-modified GfPNVs can target folate receptor-positive GL-26 brain tumors through nasal administration, and use its miR17 to downregulate the expression of MHC I on GL-26 cells, so as to activate NK cells and delay the growth of brain tumors[34]. Studies have shown that GfPNVs contain docosahexaenoic acid, which has been shown to block G1 and/or G2M cell cycle progression in leukaemia, breast, pancreatic, neuroblastoma, and colorectal cancer cells[112].

6.2. *Anti-inflammation*

Inflammation generally occurs locally, but local lesions interact with the whole body. Severe inflammation often causes systemic reactions such as fever, leukocytosis, and parenchymal organ disease. Therefore, if the inflammation is not controlled, it will develop into acute or chronic diseases, affecting the normal functioning of the body[113].

Studies have shown that PNVs derived from fruits and vegetables contain miR156a-5p, miR166a-3p, miR168a-5p, and other RNAs, which can be absorbed by mammalian intestinal epithelial cells, suggesting that the RNAs carried by PNVs can act on the gastrointestinal tract[114]. Turmeric-derived PNVs showed the potential to weaken injury factors and promote protective factors by inhibiting TNF-α, IL-6, and IL-1β and upregulating the expression of antioxidant haem oxygenase-1 (HO-1), resulting in amelioration of inflammatory symptoms and accelerated regression of colitis in mice (Fig. 4)[10]. Interestingly, turmeric-derived PNVs recovered normal levels of anti-inflammatory cytokines and myeloperoxidase in the damaged intestinal mucosa[10]. Another study showed that lipids in GgPNVs blocked NLRP3 inflammasome assembly by inhibiting downstream pathways (caspase 1 autocleavage, interleukin-1β, and IL-18 secretion, and pyroptotic cell death) of the NLRP3 inflammasome[115]. Therefore, GgPNVs are considered to be new and effective drugs to block the assembly and activation of the NLRP3 inflammasome.

The gut microbiome and its metabolites play a role in the occurrence and development of intestinal diseases by inducing inflammation and immune disorders[116]. The miRNAs in GgPNVs can promote the reproduction of Lactobacillus and affect its metabolism, thereby producing more ligands for aryl hydrocarbon receptors. Activation of aryl hydrocarbon receptors allows intestinal tissue to produce more IL-22, which in turn allows the gut to secrete more mucus and prevents bacteria from adhering to the intestinal epithelium (Fig. 5)[117]. Clostridioides difficile infection (CDI) causes antibiotic-associated colitis. Lemon-derived PNV-manipulated rhamnobacter GG and *Streptococcus thermophilus* ST-21 reduce CDI mortality by increasing bile resistance and gut survivability mediated by PNVs[118].

In addition to colitis diseases, PA and miRNA in GgPNVs can also bind to hemin-binding protein 35 (involved in biofilm development, epithelial surface colonization, and haem uptake) on the surface of *P. gingivalis*, reducing gingival periodontal pain activity and haemagglutinin expression of *P. gingivalis*, thereby alleviating symptoms of chronic periodontitis in mice[12]. Bhut Jolokia has potential anti-arthritic activity, but local adverse reactions seriously affect its availability. The Bhut Jolokia-derived PNVs (ethosomal nanovesicles) were significantly better than the commercially available capsaicin preparation Thermagel in reducing joint swelling and pain, as well as tolerance and acceptance, and have potential as a topical analgesic and antiarthritis drug[119].

6.3. *Regeneration and wound healing*

Research on PNVs in regeneration and wound healing has been extensively and deeply studied in recent years. GsPNVs deliver 20 kinds of miRNAs to BMSCs, upregulates PI3K signaling to promote nerve recovery, increase nerve regeneration by promoting the expression of neurotrophin and affecting the Ras/Erk pathway, and finally stimulate the neural differentiation, maturation, and sensory function of BMSCs. GsPNVs serve as an RNA transport platform, effectively overcoming the limitations of traditional RNA delivery strategies[7].

HaCaT cells are epidermal keratinocytes, that play an important role in wound healing. *Citrus paradisi*-derived PNVs contain antioxidant and anti-inflammatory molecules that increase HaCaT cell viability and reduce intracellular ROS generation in a dose-

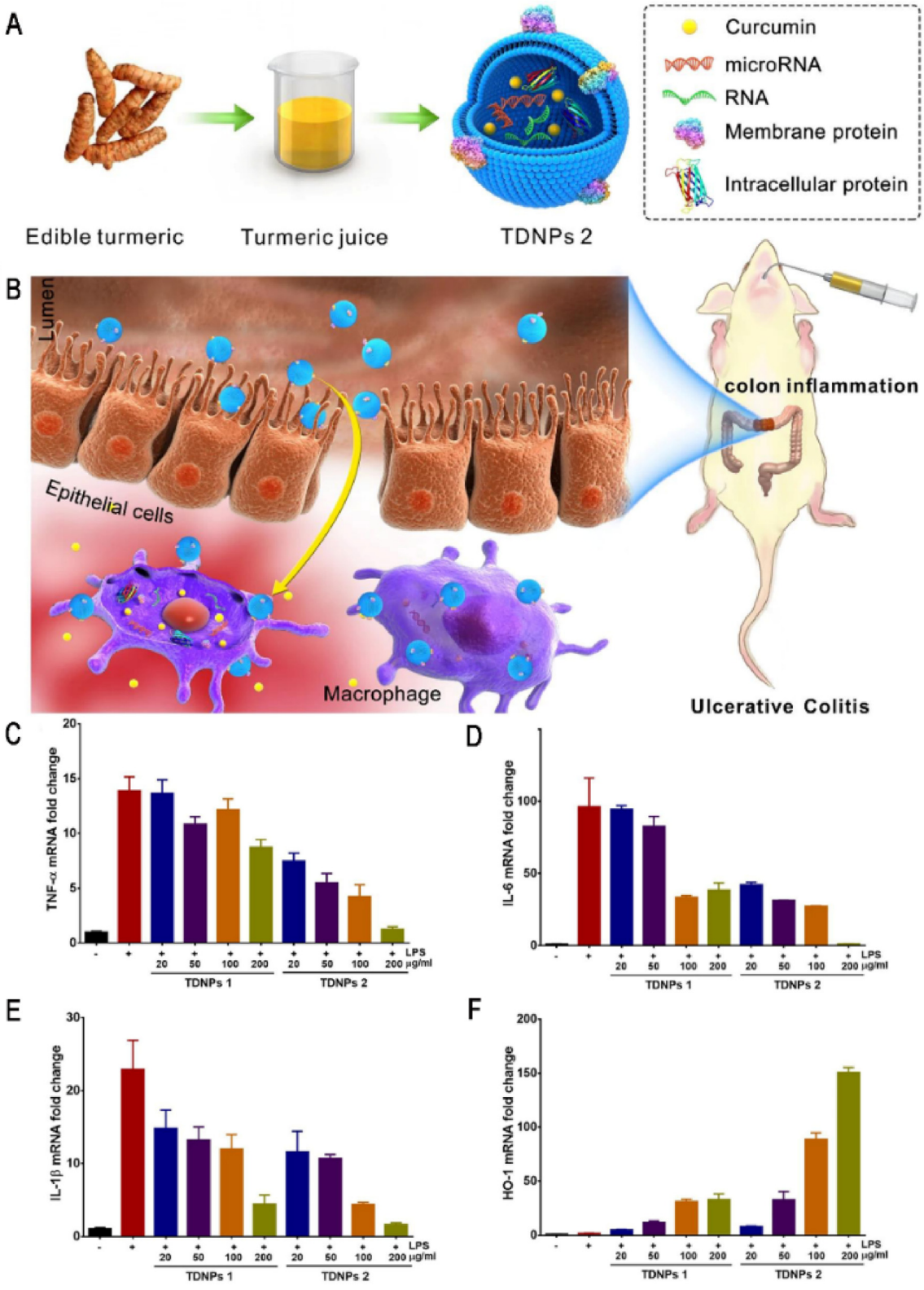

Figure 4 Turmeric-derived PNVs (TDNPs 2) in the treatment of ulcerative colitis. (A) and (B) Schematic of TDNPs 2 isolation and targeted ulcerative colitis therapy *via* oral administration. (C)−(E) mRNAs expression of pro-inflammatory cytokines, TNF-α, IL-6, and IL-1β, respectively. (F) mRNA expression of HO-1. Reprinted with permission from Ref. 10. Copyright © 2022 BioMed Central.

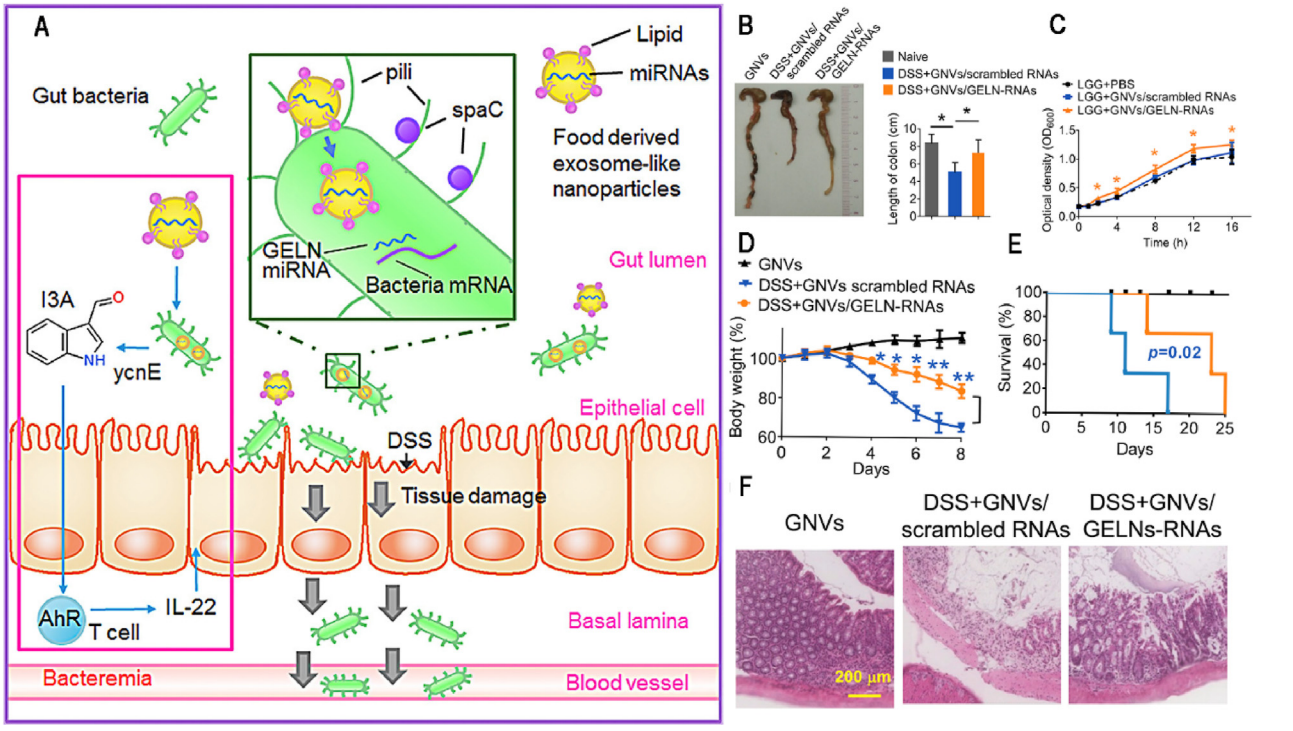

Figure 5 Ginger-derived PNVs in the treatment of colitis. (A) Schematic of the mechanism of PNVs treatment of colitis. (B)–(F) GNVs/GELN-RNAs had superior protection against DSS-induced mouse colitis. (B) Representative colons of treated mice. (C) Proliferation of LGG treated with GNVs/GELN-RNAs over time. (D) Body weight. GELN-RNAs *vs.* scrambled RNAs. (E) Survival of mice after administration of 2.5% DSS in drinking water. (F) Colon H&E-stained sections of treated mice. Reprinted with permission from Ref. 117. Copyright © 2018 Elsevier Inc.

dependent manner, which can stimulate HaCaT cell migration and increase the expression of wound-healing-related genes, proteins, and cytokines. In addition, angiogenesis is key to wound healing because nutrients are transported between cells normally when blood vessels are formed and endothelial cells are activated. *Citrus paradisi*-derived PNVs can promote the angiogenesis of human umbilical vein endothelial cells, which is a breakthrough in the treatment of skin wound healing[62]. Another study yielded similar results. Wheat grass-derived PNVs at 200 μg/mL produced amazing pro-proliferation and migration effects on the human dermal fibroblast cell line, HaCaT, and HUVECs, and increased the miRNA level of collagen type I, promoting collagen production. Meanwhile, wheat grass-derived PNVs increased the formation of endothelial tubular structures[72].

There are also studies on the role of PNVs in wound healing and regeneration from other mechanisms. Bacteria adhering to contact surfaces can lead to impaired wound healing, while the antibiofilm effect of bee-derived PNVs can be combined with their promigratory activity to treat clinical wound healing. Notably, the migration of stem cells to the wound site to assist in immune regulation, recruitment, and regeneration of fibroblasts is a critical step in wound healing, and bee-derived PNVs can be internalized by mesenchymal stem cells and facilitate their migration[120].

6.4. *Treatment of liver diseases*

PNVs have the potential to prevent and treat many types of liver diseases. MiR-4057 in honey-derived PNVs can inhibit the formation and activation of NLRP3 inflammasome in mice, and attenuate the inflammatory response caused by acute liver injury[98]. Likewise, shiitake mushroom-derived PNVs prevented the formation of inflammasomes in macrophages, thereby inhibiting the activation of the NLRP3 inflammasome, while reducing the RNA and protein levels of the Il1b gene and the secretion of interleukin-6. Interestingly, pretreatment with shiitake mushroom-derived PNVs protected mice from lipopolysaccharide (LPS) and D-galactosamine-induced liver injury[17].

LPS, one of the inducers of hepatitis and colitis, can increase the expression levels of the inflammatory factors IL-6 and IFN-γ, while garlic-derived PNV treatment inhibited the expression of these two factors in HepG2 cells. After treatment, IL-6 and IFN-γ mRNA levels were close to the baseline, indicating that garlic-derived PNVs have a strong anti-inflammatory ability[106]. In addition, the uptake of garlic-derived PNVs by HepG2 cells was positively correlated with the expression of cell surface CD98, which may be an inducible receptor for nonalcoholic fatty liver disease (NAFLD), suggesting that garlic-derived PNVs have a wide range of anti-hepatitis potential[121]. In addition to LPS, alcohol is also an important inducer of liver damage. The abundant shogaol in GgPNVs induces nuclear factor erythroid 2-related factor 2 (regulating stem cell homeostasis, drug metabolism, and antioxidant) nuclear translocation in target stem cells through the TLR4/TRIF pathway, resulting in liver detoxification/antioxidant gene expression while inhibiting ROS expression, and effectively preventing alcoholic liver injury[37].

6.5. *Anti-aging*

There is an increasing demand for antiaging products to reduce the production of melanin on the market, and plant-derived products

have become potential substitutes for chemical products because of their high content of antioxidants and few side effects[122]. GsPNVs improved the replicative senescence or senescence-related pigment phenotype of human melanocytes and skin fibroblasts by downregulating senescence-related factors and melanogenesis-associated proteins[36]. Leaf-derived PNVs of *D. morbifera* reduced the expression of melanogenesis-related genes and proteins (tyrosinase, microphthalmia-associated transcription factors TRP-1 and TRP-2) in a concentration-dependent manner. Surprisingly, *D. morbifera*-derived PNVs obtained stronger melanin inhibition than arbutin in a human epidermal model[50].

Studies have shown that the total antioxidant capacity, catalase, ascorbic acid, glutathione, and superoxide dismutase 1 contents of PNVs derived from fruit mixtures are high, and the antioxidant levels of PNVs derived from organic fruits were proven to be higher than those of PNVs from traditional agricultural sources. It is worth mentioning that after chemical and physical cleavage of fruit-derived PNVs, there is almost no change in morphology and antioxidant components, which is expected to be used as a therapeutic agent for diseases related to ageing and oxidative stress[79]. *Fragaria* × *ananassa*-derived PNVs contain a large number of anthocyanins, vitamin C, FA, and flavonols, which have a strong antioxidant capacity and can protect the response of human MSCs to oxidative stress[123]. Citrus-derived PNVs also contain vitamin C (0.009 nmol vitamin C/μg PNVs) but have a lower content than *Fragaria* × *ananassa*-derived PNVs (0.416 nmol vitamin C/μg PNVs); thus, the latter has an advantage in preventing oxidative stress in human cells[35]. Hyaluronic acid can retain water molecules, effectively inhibiting ageing. *Beta vulgaris* extract-derived PNVs promoted fibroblasts to upregulate the expression of hyaluronan synthase enzyme 2 while increasing the ratio of collagen 3 to collagen 1, which is a hallmark of anti-scarring effects[16].

6.6. The treatment of COVID-19

Since 2019, the corona virus disease 2019 (COVID-19) has swept the world. It is caused by SARS-CoV-2, which belongs to the family of coronaviruses, and there is currently no effective drug against the virus[124]. Researchers have considered the potential of PNVs for the treatment of COVID-19 because of their natural active ingredients[125]. The study identified 22 miRNAs in the GgPNVs and GfPNVs that may target the SARS-CoV-2 genome, and 11 of them showed specific targeting ability to SARS-CoV-2. Lung epithelial cells can spontaneously uptake miRNAs encapsulated by PNVs and exhibit inhibition of SARS-CoV-2 infection *in vitro* and *in vivo*[126]. In addition, studies have shown that PNVs from pear, soybean, cantaloupe, and tomato contain 6−7 miRNAs targeting SARS-CoV-2, and pea, blueberry, and grapefruit also contain 3−4 related genes. Whether these PNVs can treat COVID-19 needs to be further explored[127]. Moreover, SARS-CoV-2 Nsp12 Nsp13-derived exosomes (exosomes^{Nsp12Nsp13}) can be absorbed by pulmonary macrophages and activate nuclear factor *k*B (NF-*k*B) and a series of inflammatory factors. The miRNA alymiR396a-5p in GgPNVs can be preferentially absorbed by pulmonary epithelial cells and macrophages to eliminate exosomes^{Nsp12Nsp13}-induced pneumonia[128].

6.7. Treatment of other diseases

Oxidative stress results from an imbalance between the cellular antioxidant response and the production of ROS. Myocardial hypoxia/reoxygenation causes oxidative stress in myocardial tissue, leading to cardiovascular disease (heart failure, cardiac hypertrophy, cardiomyocyte apoptosis)[129]. Carrot-derived PNVs can effectively inhibit the production of ROS and apoptosis caused by oxidative stress in H9C2 heart-derived cells and have great potential in the treatment of diseases caused by ROS production and aberrant apoptosis including myocardial infarction[130]. Abnormal apoptosis is also a culprit in Alzheimer's and Parkinson's disease. Cabbage-derived PNVs contain anti-apoptotic components that resist aberrant apoptosis and protect cells from stress by inhibiting the activation of caspase-3[53]. Moreover, GgPNVs were easily absorbed by microglia after oral administration and inhibited the cGAS/STING/IDO1/AHR inflammatory signaling cascade, thereby improving brain inflammation and obesity symptoms in high-fat diet mice. Mice treated with PNVs exhibited enhanced insulin sensitivity and increased memory function[11]. Research on PNVs as therapeutic agents for the treatment of various diseases has endlessly emerged in recent years (Table 2[7,10−13,15−18,34,36,37,50,60,62,72,73,81,82,87,98,106,111,115,118,119,123,126,128,131−143]).

7. PNVs as nanocarriers for therapeutic agents

7.1. PNV modifications and their advantages in drug delivery

After the discovery of the cross-kingdom communication talents of PNVs (*e.g.*, anti-inflammatory, antitumor, anti-aging), more scientists have been attracted to studying their potential as DDSs[144]. Compared with artificial carriers, PNVs have the advantages of higher circulation stability, good rigidity, suitable morphology, ability to cross biological barriers, low immunogenicity, and low toxicity[3]. The clinical application of EVs to the human body requires extremely large quantities. MEVs are obtained through cell culture, which is costly in large-scale production and has a long acquisition cycle, while PNVs can be obtained in large quantities in a short period under the conditions that confer the advantages of MEVs, and have greater application potential[145].

Studies have shown that at the same lipid concentration, the effect of GgPNVs on cell proliferation was significantly lower than that of commercially available cationic liposomes[146]. Moreover, under the acidic pH condition of the tumor microenvironment, the drug release efficiency of GgPNVs was faster than that of commercial liposomes, which laid a solid foundation for their use as a new generation of drug delivery carriers. PNVs can bind to hydrophobic agents such as FA, curcumin, and zymosan A without altering their biological activities[147]. After modification with FA, the fluorescence intensity of PNVs in tumors was increased by 2.8-fold, and the Ki67-positive cells were reduced from 64.67% to 10%, showing stronger antitumor efficacy[146]. In addition, PNVs also possess unique targeting capabilities. Garlic-derived PNVs can be preferentially and selectively taken up by microglia in HFD mice and further inhibit the activation of cells, which provides hope for their development as excellent therapeutic carriers for brain diseases[11]. The relevant studies of PNVs as DDSs are shown in Table 3[14,53,61,77,146,148−150].

7.2. PNVs as drug delivery carriers

7.2.1. Delivery of therapeutic drugs

Drug therapy still lacks vectors that can efficiently transfect target cells without generating host immune responses, while PNVs are expected to compensate for the shortcomings of current drug carriers because of the above advantages. The *in vivo*

Table 2 Plant-derived nanovesicles as therapeutic agents.

Source	Sample	Application	Biological function	Ref.
Ginseng	Root juice	Anti-aging	Improved the replicative senescent or senescence-associated pigmented phenotypes	36
		Regeneration	Stimulated the neural differentiation, maturation, and sensory function acquisition of BMSCs	7
		Anti-melanoma	Promoted the polarization of M2 to M1 phenotype and produce total reactive oxygen species	87
		Anti-tumor	Enhanced PD-1 mAb anti-tumor efficacy in activating tumor-infiltrated T lymphocytes	131
Grapefruit	Fruit juice	Wound healing	Increased cell viability and cell migration while reducing intracellular ROS production in a dose-dependent manner	62
		Anti-melanoma	Caused cell cycle arrest at the G2/M checkpoint	132
		Anti-brain tumor	Delivered miR17 to the brain and is selectively taken up by GL-26 tumor cells	34
		Anti-liver metastasis of colon cancer	Acted as an inhibitor for liver metastasis through induction of M1 macrophages	133
		Anti-COVID-19	Targeted SARS-CoV-2 genome to inhibit SARS-CoV-2 infection	126
Grape	Fruit juice, seed juice	Anti-oxidative	Counteracted the oxidative damages induced in Caco-2 cells by using hydrogen peroxide	134
		Anti-colitis	Mediated intestinal remodeling and prevention of colitis induced by sodium dextran sulfate	135
Blueberry	Fruit juice	Protect vascular system	Inhibited TNF-α-induced ROS generation, and modulated TNF-α-induced expression of 29 genes	18
Strawberry	Fruit juice		Identified homologous sequences Fra a 1 and Fra a 4 of strawberry allergens	136
	Fruit juice	Anti-oxidative stress	Protected the response of human MSCs to oxidative stress	123
Lemon	Fruit juice	Protect gut bacteria	Restricted Msp1 and Msp3 production and reduced bile accessibility to cell membranes	137
		Anti-gastric cancer	Mediated ROS production and exhibited anticancer activity	73
		Anti-colitis	Mediated bile resistance, increased gut survival, and decreased CDI mortality	118
Citrus	Fruit juice	Anti-inflammation	Limited inflammatory stimuli and restored functional barrier by increasing the tight junction OCLN protein	138
		Reverse diet-induced gut modifications	Reduced plasma lipids and inflammation in gastrointestinal disease	139
Asparagus cochinchinensis (Lour.) Merr.	Root juice	Anti-hepatocellular carcinoma	Inhibited tumor growth without side effects	81
Bitter melon	Fruit juice	Anti-oral squamous cell carcinoma	Reduced oral squamous cell carcinoma resistance to 5-FU by downregulating NLRP3	111
Tea flowers	Flower juice	Anti-metastatic breast cancer	Induced mitochondrial damage, cell cycle arrest, and eventually lead to cancer cell apoptosis	13
Garlic	Stem juice	Reverse high-fat diet induced obesity	Taken up by microglia and inhibited brain inflammation in high-fat diet (HFD) mice	11
			Treated diseases associated with high CD98 expression	106
Turmeric	Stem juice	Anti-colitis	Regulated the expression of pro-inflammatory cytokines and antioxidant genes, and promoted colitis resolution	10

(*continued on next page*)

Table 2 (*continued*)

Source	Sample	Application	Biological function	Ref.
Ginger	Rhizome juice, root juice	Anti-inflammation	Internalized by intestine cells, and counteract LPS-induced inflammation by downregulating NF-κB, IL-6, IL-8, and TNF-α expression	60
		Anti-inflammation	Inhibited NLRP3 inflammasome activation	115
		Anti-COVID-19	Targeted SARS-CoV-2 genome to inhibit SARS-CoV-2 infection	126
		Anti-COVID-19	Induced TNF-α, IL-6, and IL-1b to promote lung epithelial cell apoptosis	128
		Prevent/treat chronic periodontitis	Reduced the pathogenic mechanism of *Porphyromonas gingivalis*	12
		Protect the injured liver	Induced the expression of detoxification/ antioxidation genes in the liver and inhibited the production of ROS	37
		Anti-tumor	Inhibited tumor growth in xenograft models	140
Honey	Honey	Anti-inflammation	Inhibited NLRP3 inflammasome activation	98
Propolis	Propolis	Anti-lung cancer	Exhibited high cytotoxicity against lung cancer cells	141
Mulberry bark	Bark juice	Anti-colitis	Conferred protection against colitis by promoting Hsp70 member 8-mediated activation of the AhR signaling pathway	142
Broccoli	Fruit juice	Anti-colitis	Mediated the activation of adenosine monophosphate-activated protein kinase in DCs to prevent DCs activation	82
Beta vulgaris	Root juice	Anti-aging	Promoted angiogenesis and the anti-aging and anti-scarring abilities of fibroblasts	16
Corn	Fruit juice	Anti-tumor	Inhibited the proliferation of colon 26 cells and through TNF-α production by activation of macrophages	15
Carrot	Root juice	Anti-oxidative	Inhibited the reduction in the expression of antioxidant molecules, including Nrf-2, HO-1, and NQO-1	130
Shiitake mushroom	Fungus	Protect the injured liver	Inhibited NLRP3 inflammasome activation	17
Dendropanax morbifera	Leaf and stem juice	Anti-melanoma	Reduced melanin content and tyrosinase activity in a concentration-dependent manner	50
Wheat	Fruit juice	Wound healing	Promoted the proliferation and migration of endothelial cells, epithelial cells, and dermal fibroblasts	72
Bhut Jolokia	Fruit juice	Anti-arthritis	Relieved the joint swelling and pain of the rat model	119
Calotropis gigantea	Leaf juice	Regulate brain nerves	Facilitated the early development of cytoarchitecture	143

BMSCs, bone marrow mesenchymal stem cells; CDI, clostridioides difficile infection; COVID-19, corona virus disease 2019; DCs, dendritic cells; HO-1, haem oxygenase-1; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α.

circulation time, tissue penetration ability and specificity of the carrier, and the efficient release of the encapsulated material are the keys to its anchoring to a specific tissue[151]. GfPNVs can stably circulate in the peripheral blood of tumor-bearing mice for 5 days, and activated leukocyte membrane-modified GfPNVs can carry doxorubicin and curcumin to precisely target breast tumors and inflamed colon, thus inhibiting disease progression[149]. Another study used GfPNVs encapsulated methotrexate (MTX) to treat colitis, and the protection of PNVs significantly reduced the side effects of MTX[150]. AvPNVs can protect indocyanine green (ICG) from degradation and still have a retention rate of more than 90% after 30 days of drug loading. AvPNVs contain Cer and GlcCer (accounting for 58.53% of the total lipids), which are responsible for maintaining the water permeability barrier. They promote the penetration of AvPNVs penetrating the cuticle barrier to the dermis to deliver ICG, thereby effectively inhibiting the growth of melanoma. Therefore, compared with liposomes, AvPNVs have better transdermal properties and are expected to become novel noninvasive drug carriers for the treatment of skin cancer (Fig. 6)[14]. GgPNVs encapsulated mesoporous silica nanoparticles loaded with TNF-α antibody, which ensured the stability of the antibody in the intestine during the delivery process, and targeted delivery to the colon. After passing through the intestinal epithelium, the antibody is released in the lamina propria to achieve a high accumulation of antibody in the inflamed colon, which can effectively prevent and treat inflammatory bowel disease (IBD)[152]. At the same time, GgPNVs effectively blocked the inflammation caused by the NLRP3 inflammasome and exerted a multipathway synergistic and effective anti-colitis effect[148].

Table 3 Plant-derived nanovesicles as delivery carriers for disease therapeutics.

Carrier	Drug	Application	Advantage	Ref.
Cabbage	miR-184, doxorubicin	Tumor	Low cytotoxicity, multiple functions, low production cost, and high production yield	53
Acerola	miRNA	Digestive system diseases	Nucleic acids can be directly encapsulated without any reagents and protected from acids and bases	61
Ginger	Infliximab	Colitis	Stability in the gastrointestinal tract, colon-targeted delivery, and high intestinal epithelium permeability	148
	Doxorubicin	Colon cancer	GgPNVs have better pH-dependent drug release ability than commercial liposomes	146
Aloe vera	Indocyanine green	Melanoma	AvPNVs can be efficiently absorbed by melanoma cells with good structure, antioxidant capacity, and stability	14
Grapefruit	Doxorubicin, curcumin	Tumor, colitis	Production of GfPNVs is easily scaled up and the GfPNVs can be coated with leukocyte membranes from an individual	149
	Methotrexate	Colitis	GfPNVs are easily absorbed by intestinal macrophages and improve dextran sulfate-induced colitis in mice	150
	Curcumin	Tumor	GfPNVs are more stable than cationic liposomes and can maintain the biological activity of curcumin	77

AvPNVs, aloe vera-derived PNVs; GfPNVs, grapefruit-derived PNVs; GgPNVs, ginger-derived PNVs.

7.2.2. *Delivery of genetic drugs*

In plants lacking a specific immune system, RNAs are the key defence mechanisms to help plants defend against bacteria, fungi, and viruses, while PNVs are the main carriers to help RNA function between plants and animals[153]. Studies have shown that small RNAs (especially miRNAs) are easy to be encapsulated in PNVs because of their size advantages. MiR-184 is widely involved in biological and pathological processes such as apoptosis and tumorigenesis. Cabex-derived PNVs encapsulated miR-184 with little influence on potential and morphology, and could efficiently deliver miR-184 to colon cancer cells, increasing the level of miR in cells by 246,000-fold[53]. In another study, GfPNVs loaded with miR-18a preferentially targeted liver macrophages, promoted their polarization to M1 macrophages, and then produced IL-12, which stimulated liver immune cells such as natural killer (NK) and natural killer T (NKT) cells, thus inhibiting liver metastasis of colon cancer. This strategy can also be applied to the treatment of liver metastases from various cancers[133]. MiRNAs encapsulated by acerola fruit-derived PNVs were hardly affected by ribonucleases, acids, and alkalis, and obtained greater protection than those encapsulated in milk-derived vesicles. In addition, acerola fruit-derived PNVs can directly encapsulate nucleic acids by simply incubating them on ice without the need for other special reagents. After oral administration, PNVs enter the blood vessels from the intestinal tract and reach everywhere through the blood circulation, which is expected to become a new carrier for carrying nucleic acids[61,154]. Free siRNA-CD98 can only stay in the stomach, while GgPNVs encapsulating siRNA-CD98 can carry it to the stomach, ileum, and colon, and are highly retained in the colon and ileum, reducing the expression of CD98 in the colon, thus controlling the development of colitis and related cancer. It is worth noting that the effective dose of GgPNVs carrying siRNA-CD98 is approximately 10,000 times lower than that of free siRNA-CD98[155]. GfPNVs can also carry luciferase siRNA to inhibit the expression of the luciferase reporter gene in GL26-Luc and A549-Luc cells[77].

8. Toxicity and immunogenicity

Synthetic nanoscale delivery systems, such as liposomes[156], polymer nanoparticles[157], micelles[158], and metal nanoparticles[159] can enhance the efficacy of drug therapy and improve bioavailability. However, synthetic materials will remain in the liver and spleen in large quantities, causing systemic toxicity. To improve bioavailability, stability, and tissue distribution; reduce toxicity; prevent physical and chemical degradation; and prolong the action time of drugs, scientists have turned their focus to biomimetic DDSs, which are characterized by higher safety and lower immunogenicity. Regarding safety, the various components in PNVs come from cells and do not harbour any zoonotic or human pathogens, so the immune risk *in vivo* is lower than that of MEVs[147]. Compared with liposome, the cell survival rate of colon-26 and RAW264.7 cells treated with GgPNVs for 24 h (the highest concentration was 200 μmol/L) changed little. *In vivo* experiments showed the same results, and there was almost no difference in hepatic and renal functional parameters, white blood cells, and red blood cells of mice in the GgPNVs and PBS-treated group[155]. This proves that PNVs are non-toxic compared with commercially available liposomes and have the potential to replace them as drug delivery vehicles.

The route of administration affects the safety of PNVs *in vivo*. TfPNVs activated the complement system after i.v. injection, causing the liver and spleen to become heavier and potentially hepatotoxic, while the organ weight, body weight, and toxicity of the oral route group were negligible. It may be that the i.v. administration of TfPNVs triggered the immune response and induced hepatotoxicity, while the oral TfPNVs were degraded by the gastrointestinal tract, which was not retained by filter organs, and the immunogenicity was extremely low[13]. On the contrary, AvPNVs were not toxic to red blood cells after i.v. injection, and the results of tissue sections of mice showed that there was no obvious damage to various organ[14]. Customized experiments tend to have clear objectives and narrow research scope. The challenges related to biosafety and toxicity of different drug delivery routes and unknown

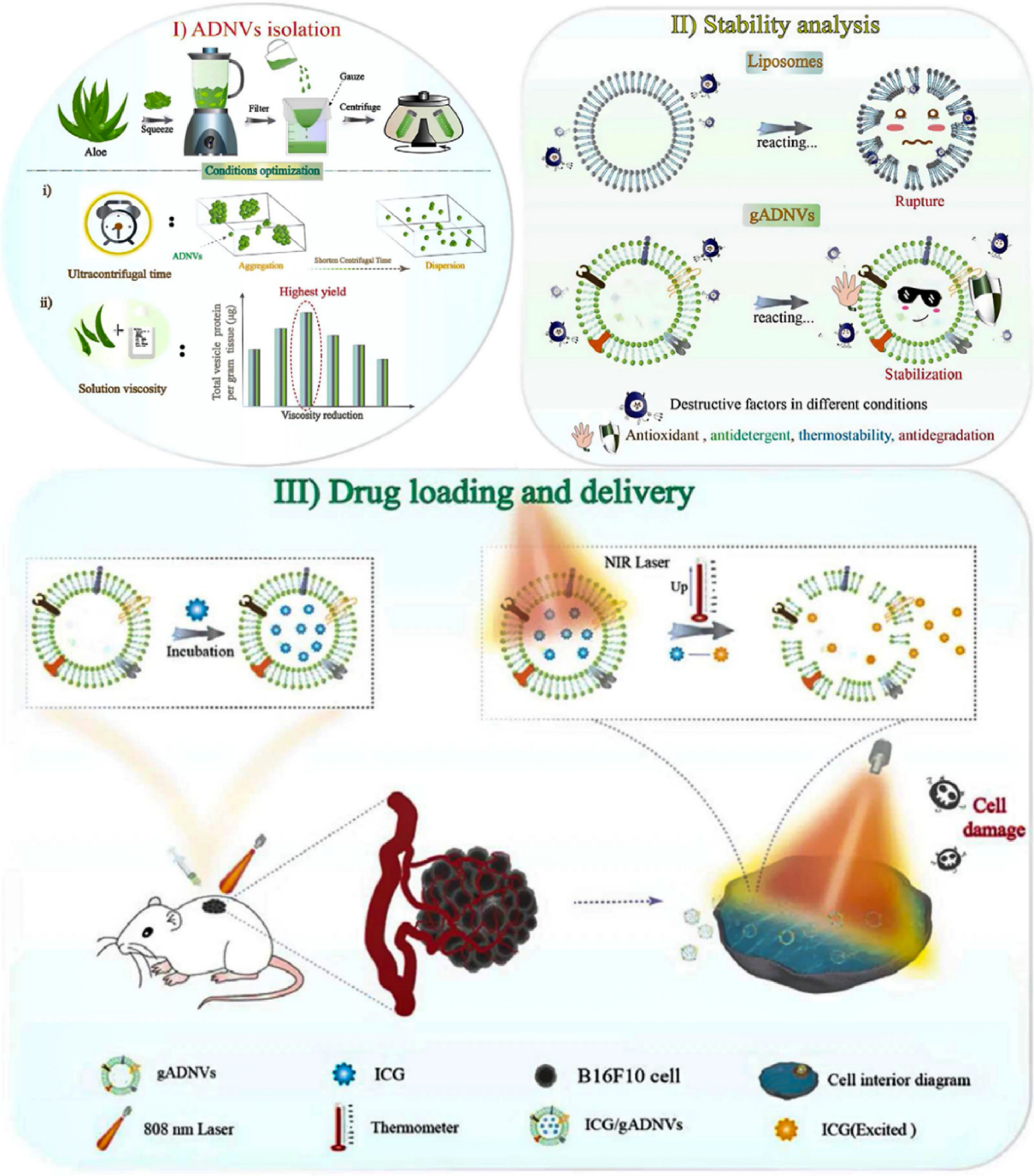

Figure 6 Schematic of aloe vera-derived PNVs isolation, purification, and drug delivery; PNVs loaded with photosensitizers have good stability for cancer therapy. Reprinted with permission from Ref. 14. Copyright © 2021 BioMed Central.

bioactive components of PNVs are still incomplete, which represents a valuable field related to human health.

9. Clinical trials

Currently, relevant clinical experiments have proven the application value of PNVs[160]. A study used grape-derived PNVs as anti-inflammatory agents to prevent oral mucositis during radiotherapy and chemotherapy in patients with head and neck tumors, and evaluated their effects on cytokine production and tumor-related immune responses in patients. Sixty subjects were given daily oral administration of grape-derived PNVs for 35 days to assess their efficacy. The results of phase I clinical trial were obtained in June 2022 (ClinicalTrials.gov: NCT01668849). The drug loading capacity of PNVs as carriers was also investigated. Taking advantage of the strong combination of hydrophobic drugs and exosomes, the researchers loaded curcumin into PNVs to overcome the administration barrier of curcumin as an anti-inflammatory agent, increase its stability and solubility, and improve the limited bioavailability of high-dose curcumin. Currently, the study has successfully recruited 35 subjects and is in phase I clinical trial stage, which is expected to end by the end

of 2022 (ClinicalTrials.gov: NCT01294072)[161]. Regrettably, a clinical study examining GgPNVs and AvPNVs-attenuating insulin resistance and chronic inflammation in patients with polycystic ovary syndrome was withdrawn because the researchers left the university before the project was approved (ClinicalTrials.gov: NCT03493984).

10. Challenges and perspectives

Plants provide a valuable resource for drug development and have been studied in traditional medical practice for thousands of years. Since the role of PNVs was discovered, it became a critical and rapidly developing field in the scientific community. PNVs carry a variety of proteins, nucleic acids, lipids, and other plant-specific natural chemicals, with antitumor, anti-inflammatory, wound healing, regeneration, and antiaging properties, and they can be used in the treatment of liver disease and COVID-19. Based on the characteristics of wound healing, regeneration, anti-inflammation, and antioxidation, PNVs are expected to become functional DDS for the treatment of chronic diseases[162]. In addition, the ability of PNVs to regulate the tumor microenvironment makes it valuable for in-depth analysis in the field of antitumor. PNVs mediate the transboundary gene regulation of plants and animals, which enables RNA in plants to survive in an active form in animals, and can regulate the biological process of host cells. Therefore, PNVs cannot induce the immune response of normal host cells, have high biocompatibility and safety, and can minimize cytotoxicity. Based on their original function, PNVs can resist the action of digestive enzymes, protect their contents from degradation, and deliver the active ingredients to the site of action through the blood−brain barrier without passing through the placenta. This feature also renders them safe to use in the treatment of diseases in pregnant women[78,163]. Most significantly, PNVs are available in abundant sources and large quantities, allowing researchers to select suitable species from a wide range according to disease adaptation, reducing the cost of time and capital to a certain extent. Moreover, in addition to fresh plants, active PNVs can also be obtained from dried or even decocted plants, which sheds light on new sources of PNVs.

After the discovery of the function of PNVs in mediating information transfer and material exchange between species, it speedily became the forefront of the DDSs field. In recent years, scientists have aimed to compare the pros and cons of PNVs with MEVs and synthetic nanoparticles to gain insight into the potential and advantages of PNVs as DDS[164]. As a DDS, PNVs currently have three modification techniques: (1) Biomolecules are encapsulated in the lipid layer of PNVs or the nucleus, so that the drug can safely reach the target site. (2) Biomolecules are modified on the surface of PNVs to improve their targeting. (3) Nanoparticles encapsulating biomolecules are loaded into PNVs to facilitate targeted drug delivery and prevent premature leakage. Analogous to MEVs, when PNVs used as drug carriers, the limited drug load is also one of the key issues that limit its clinical transformation. Membrane fusion technology is designed to give carriers the advantages of two or more membranes to meet the expectations of researchers for new carriers[165,166]. Therefore, we believe that it is feasible for PNVs to fuse with liposomes to improve drug loading and long-circulation stability, which will be the focus of exploration in the future. In addition, the interaction mechanism between PNVs as drug carriers and receptor cells has not been concluded, and the discussion of this issue should start from the internalization mechanism and physiological and biochemical characteristics of PNVs. Finally, the interaction between loaded drugs and innate active ingredients of PNVs has not been reported in the literature. Before designing an effective strategy, the contents of PNVs can be removed in advance, but it is strongly recommended that the shape, size, and activity of the contents be verified again after removal to ensure that the unloading process does not affect the original properties of the vesicle.

Scientists' in-depth study of the role of PNVs has tapped their potential as a therapeutic agent for the next generation of human diseases. However, to realize its clinical transformation, we must address unsolved problems related to sourcing, biogenesis, isolation, purification, storage, and characterization. The sources of PNVs are extensive and the cost is low, but the sources are affected by seasons and regions. Though tissue culture technology is a scheme to provide stable plant sources, it has not been popularized in the existing research on PNVs. The contents of PNVs vary greatly depending on plant parts and sources. For example, the contents of PNVs from roots, stems, and leaves of the same plant may vary greatly, and the antioxidant components of PNVs from organic fruits and vegetables are significantly higher than those from traditional agriculture[19,79]. Therefore, a case-by-case analysis is needed and it is recommended to add activity evaluation into the quality control system of PNVs to ensure that more detailed and convincing data can be obtained. The mechanism by which PNVs are sorted into various types of vesicles has not been elucidated, for example, EXPO is one of the formation pathways of EVs, but its biogenesis is still unknown. The in-depth study of PNVs began later than that of MEVs, but their isolation methods are similar. However, PNVs are more complex and can be obtained from seeds, roots, stems, leaves, fruits, and other parts. Therefore, PNVs often require tailor-made pretreatment and isolation and purification methods, along with the development of a repeatable preparation process to support their medical applications[167]. The storage method after isolation is also an important step in the clinical development of PNVs. Determining how to solve the problem of the impact of cryogenic freezing on the structure of PNVs and identify high-quality cryoprotectants to prolong the retention time of PNVs are the keys to efficient storage of PNVs[168]. Trehalose is a commonly used cryoprotectant for MEVs, but the types of cryoprotectants suitable for PNVs to maintain their composition and structure stability still need further exploration[169]. Besides, MEVs still maintain a nearly spherical shape under aerosol desiccation, but whether this method can maintain the activity of PNV content substances is still not verified by experiments[170]. Moreover, PNVs do not come from biological fluid and lack specific biomarkers, so their targeting *in vivo* may be worse than that of MEVs, and they can only be endowed with targeting ability by exogenous modification[171]. However, targeted ligands increase unpredictability while changing the distribution trend of PNVs, so the progress of this work requires strict determination of isolation, quantification, morphological characterization methods, and *in vivo* transport pathways.

Research on PNVs is still in its infancy in the entire field of DDSs. However, in recent years, this research has expanded to involve molecular biology, pharmaceutics, biomedicine, materials science, and immunology, and has combined nanotechnology and drug delivery technology. Scientists are maximizing the development of PNVs, accelerating the pace of their clinical translation, and opening a new chapter in the treatment of human diseases.

Acknowledgments

This work was supported by National Natural Science Foundation of China (82274103, 82074272, China), Program of Shanghai Academic Research Leader (21XD1403400, China), the Science and Technology Commission of Shanghai Municipality (20S21900300, China), and Shanghai Sailing Program (20YF1412100, China).

Author contributions

The manuscript of this review was composed by Aixue Li, Dan Li, Yongwei Gu, Rongmei Liu, Xiaomeng Tang, Yunan Zhao, and Fu Qi under the guidance and advice of Jiyong Liu and Jifu Wei. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Li AX, Zhao YN, Li YX, Jiang LD, Gu YW, Liu JY. Cell-derived biomimetic nanocarriers for targeted cancer therapy: cell membranes and extracellular vesicles. *Drug Deliv* 2021;**28**:1237−55.
2. Akhmerov A, Parimon T. Extracellular vesicles, inflammation, and cardiovascular disease. *Cells* 2022;**11**:2229.
3. Cheng L, Hill AF. Therapeutically harnessing extracellular vesicles. *Nat Rev Drug Discov* 2022;**21**:379−99.
4. Zhou QF, Ma K, Hu HH, Xing XL, Huang X, Gao H. Extracellular vesicles: their functions in plant-pathogen interactions. *Mol Plant Pathol* 2022;**23**:760−71.
5. Duan L, Xu LM, Xu X, Qin Z, Zhou XY, Xiao Y, et al. Exosome-mediated delivery of gene vectors for gene therapy. *Nanoscale* 2021; **13**:1387−97.
6. Nguyen J, Fuhrmann G. Extracellular vesicles − a versatile biomaterial. *Adv Healthc Mater* 2022;**11**:e2200192.
7. Xu XH, Yuan TJ, Dad HA, Shi MY, Huang YY, Jiang ZH, et al. Plant exosomes as novel nanoplatforms for microRNA transfer stimulate neural differentiation of stem cells *in vitro* and *in vivo*. *Nano Lett* 2021;**21**:8151−9.
8. Suharta S, Barlian A, Hidajah AC, Notobroto HB, Ana ID, Indariani S, et al. Plant-derived exosome-like nanoparticles: a concise review on its extraction methods, content, bioactivities, and potential as functional food ingredient. *J Food Sci* 2021;**86**:2838−50.
9. Regente M, Corti-Monzon G, Maldonado AM, Pinedo M, Jorrin J, de la Canal L. Vesicular fractions of sunflower apoplastic fluids are associated with potential exosome marker proteins. *FEBS Lett* 2009; **583**:3363−6.
10. Liu C, Yan XJ, Zhang YJ, Yang M, Ma YN, Zhang YY, et al. Oral administration of turmeric-derived exosome-like nanovesicles with anti-inflammatory and pro-resolving bioactions for murine colitis therapy. *J Nanobiotechnol* 2022;**20**:206.
11. Sundaram K, Mu JY, Kumar A, Behera J, Lei C, Sriwastva MK, et al. Garlic exosome-like nanoparticles reverse high-fat diet induced obesity *via* the gut/brain axis. *Theranostics* 2022;**12**:1220−46.
12. Sundaram K, Miller DP, Kumar A, Teng Y, Sayed M, Mu JY, et al. Plant-derived exosomal nanoparticles inhibit pathogenicity of *Porphyromonas gingivalis*. *iScience* 2019;**21**:308−27.
13. Chen QB, Li Q, Liang YQ, Zu MH, Chen NX, Canup BSB, et al. Natural exosome-like nanovesicles from edible tea flowers suppress metastatic breast cancer *via* ROS generation and microbiota modulation. *Acta Pharm Sin B* 2022;**12**:907−23.
14. Zeng LP, Wang HY, Shi WH, Chen LF, Chen TT, Chen GY, et al. Aloe derived nanovesicle as a functional carrier for indocyanine green encapsulation and phototherapy. *J Nanobiotechnol* 2021;**19**: 439.
15. Sasaki D, Kusamori K, Takayama Y, Itakura S, Todo H, Nishikawa M. Development of nanoparticles derived from corn as mass producible bionanoparticles with anticancer activity. *Sci Rep* 2021;**11**:22818.
16. Mahdipour E. Beta vulgaris juice contains biologically active exosome-like nanoparticles. *Tissue Cell* 2022;**76**:101800.
17. Liu BL, Lu YZ, Chen XQ, Muthuraj PG, Li XZ, Pattabiraman M, et al. Protective role of shiitake mushroom-derived exosome-like nanoparticles in D-galactosamine and lipopolysaccharide-induced acute liver injury in mice. *Nutrients* 2020;**12**:477.
18. De Robertis M, Sarra A, D'Oria V, Mura F, Bordi F, Postorino P, et al. Blueberry-derived exosome-like nanoparticles counter the response to TNF-alpha-induced change on gene expression in EA.hy926 cells. *Biomolecules* 2020;**10**:742.
19. Cui Y, Gao JY, He YL, Jiang LW. Plant extracellular vesicles. *Protoplasma* 2020;**257**:3−12.
20. Anusha R, Priya S. Dietary exosome-like nanoparticles: an updated review on their pharmacological and drug delivery applications. *Mol Nutr Food Res* 2022;**66**:e2200142.
21. Pinedo M, de la Canal L, de Marcos Lousa C. A call for rigor and standardization in plant extracellular vesicle research. *J Extracell Vesicles* 2021;**10**:e12048.
22. Fang Y, Wang ZW, Liu XL, Tyler BM. Biogenesis and biological functions of extracellular vesicles in cellular and organismal communication with microbes. *Front Microbiol* 2022;**13**: 817844.
23. van Niel G, Carter DRF, Clayton A, Lambert DW, Raposo G, Vader P. Challenges and directions in studying cell−cell communication by extracellular vesicles. *Nat Rev Mol Cell Biol* 2022;**23**: 369−82.
24. An QL, Ehlers K, Kogel KH, van Bel AJ, Hückelhoven R. Multivesicular compartments proliferate in susceptible and resistant MLA12-barley leaves in response to infection by the biotrophic powdery mildew fungus. *New Phytol* 2006;**172**:563−76.
25. Cai Q, He BY, Jin HL. A safe ride in extracellular vesicles − small RNA trafficking between plant hosts and pathogens. *Curr Opin Plant Biol* 2019;**52**:140−8.
26. Cai Q, Qiao LL, Wang M, He BY, Lin FM, Palmquist J, et al. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* 2018;**360**:1126−9.
27. Poulsen CP, Dilokpimol A, Mouille G, Burow M, Geshi N. Arabinogalactan glycosyltransferases target to a unique subcellular compartment that may function in unconventional secretion in plants. *Traffic* 2014;**15**:1219−34.
28. Cai Q, He BY, Wang SM, Fletcher S, Niu DD, Mitter N, et al. Message in a bubble: shuttling small RNAs and proteins between cells and interacting organisms using extracellular vesicles. *Annu Rev Plant Biol* 2021;**72**:497−524.
29. Bruns C, McCaffery JM, Curwin AJ, Duran JM, Malhotra V. Biogenesis of a novel compartment for autophagosome-mediated unconventional protein secretion. *J Cell Biol* 2011;**195**:979−92.
30. Di Bella MA. Overview and update on extracellular vesicles: considerations on exosomes and their application in modern medicine. *Biology (Basel)* 2022;**11**:804.
31. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;**7**: 1535750.
32. Chen JC, Li PL, Zhang TY, Xu ZP, Huang XW, Wang RM, et al. Review on strategies and technologies for exosome isolation and purification. *Front Bioeng Biotechnol* 2022;**9**:811971.
33. Ströhle G, Gan J, Li H. Affinity-based isolation of extracellular vesicles and the effects on downstream molecular analysis. *Anal Bioanal Chem* 2022;**414**:7051−67.

34. Zhuang XY, Teng Y, Samykutty A, Mu JY, Deng ZB, Zhang LF, et al. Grapefruit-derived nanovectors delivering therapeutic miR17 through an intranasal route inhibit brain tumor progression. *Mol Ther* 2016;**24**:96−105.
35. Baldini N, Torreggiani E, Roncuzzi L, Perut F, Zini N, Avnet S. Exosome-like nanovesicles isolated from *Citrus limon* L. exert antioxidative effect. *Curr Pharm Biotechnol* 2018;**19**:877−85.
36. Cho EG, Choi SY, Kim H, Choi EJ, Lee EJ, Park PJ, et al. *Panax ginseng*-derived extracellular vesicles facilitate anti-senescence effects in human skin cells: an eco-friendly and sustainable way to use ginseng substances. *Cells* 2021;**10**:486.
37. Zhuang XY, Deng ZB, Mu JY, Zhang LF, Yan J, Miller D, et al. Ginger-derived nanoparticles protect against alcohol-induced liver damage. *J Extracell Vesicles* 2015;**4**:28713.
38. Huang YF, Wang SM, Cai Q, Jin HL. Effective methods for isolation and purification of extracellular vesicles from plants. *J Integr Plant Biol* 2021;**63**:2020−30.
39. Liu Y, Wu S, Koo Y, Yang A, Dai YW, Khant H, et al. Characterization of and isolation methods for plant leaf nanovesicles and small extracellular vesicles. *Nanomedicine* 2020;**29**:102271.
40. He BY, Cai Q, Qiao LL, Huang CY, Wang SM, Miao WL, et al. RNA-binding proteins contribute to small RNA loading in plant extracellular vesicles. *Nat Plants* 2021;**7**:342−52.
41. Gardiner C, Di Vizio D, Sahoo S, Théry C, Witwer KW, Wauben M, et al. Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey. *J Extracell Vesicles* 2016;**5**:32945.
42. Crescitelli R, Lässer C, Lötvall J. Isolation and characterization of extracellular vesicle subpopulations from tissues. *Nat Protoc* 2021;**16**:1548−80.
43. Nemidkanam V, Chaichanawongsaroj N. Characterizing *Kaempferia parviflora* extracellular vesicles, a nanomedicine candidate. *PLoS One* 2022;**17**:e0262884.
44. Usui K, Yamamoto H, Oi T, Taniguchi M, Mori H, Fujita Y. Extracellular vesicle-mediated secretion of protochlorophyllide in the cyanobacterium *leptolyngbya boryana*. *Plants (Basel)* 2022;**11**:910.
45. Chen BY, Sung CW, Chen C, Cheng CM, Lin DP, Huang CT, et al. Advances in exosomes technology. *Clin Chim Acta* 2019;**493**:14−9.
46. Konoshenko MY, Lekchnov EA, Vlassov AV, Laktionov PP. Isolation of extracellular vesicles: general methodologies and latest trends. *BioMed Res Int* 2018;**2018**:8545347.
47. Yu XL, Chen XH, Sun ZX, Niu RY, Deng Y, Wang L, et al. Ultracentrifugation-free enrichment and quantification of small extracellular vesicles. *Anal Chem* 2022;**94**:10337−45.
48. Phillips W, Willms E, Hill AF. Understanding extracellular vesicle and nanoparticle heterogeneity: novel methods and considerations. *Proteomics* 2021;**21**:e2000118.
49. Liangsupree T, Multia E, Riekkola ML. Modern isolation and separation techniques for extracellular vesicles. *J Chromatogr A* 2021;**1636**:461773.
50. Lee R, Ko HJ, Kim K, Sohn Y, Min SY, Kim JA, et al. Anti-melanogenic effects of extracellular vesicles derived from plant leaves and stems in mouse melanoma cells and human healthy skin. *J Extracell Vesicles* 2019;**9**:1703480.
51. Monguió-Tortajada M, Gálvez-Montón C, Bayes-Genis A, Roura S, Borràs FE. Extracellular vesicle isolation methods: rising impact of size-exclusion chromatography. *Cell Mol Life Sci* 2019;**76**:2369−82.
52. Martínez-Greene JA, Hernández-Ortega K, Quiroz-Baez R, Resendis-Antonio O, Pichardo-Casas I, Sinclair DA, et al. Quantitative proteomic analysis of extracellular vesicle subgroups isolated by an optimized method combining polymer-based precipitation and size exclusion chromatography. *J Extracell Vesicles* 2021;**10**:e12087.
53. You JY, Kang SJ, Rhee WJ. Isolation of cabbage exosome-like nanovesicles and investigation of their biological activities in human cells. *Bioact Mater* 2021;**6**:4321−32.
54. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option? *Int J Mol Sci* 2020;**21**:6466.
55. Lane RE, Korbie D, Trau M, Hill MM. Purification protocols for extracellular vesicles. *Methods Mol Biol* 2017;**1660**:111−30.
56. Yousif G, Qadri S, Parray A, Akhthar N, Shuaib A, Haik Y. Exosomes derived neuronal markers: immunoaffinity isolation and characterization. *NeuroMol Med* 2022;**24**:339−51.
57. Patel GK, Khan MA, Zubair H, Srivastava SK, Khushman M, Singh S, et al. Comparative analysis of exosome isolation methods using culture supernatant for optimum yield, purity and downstream applications. *Sci Rep* 2019;**9**:5335.
58. Tiwari S, Kumar V, Randhawa S, Verma SK. Preparation and characterization of extracellular vesicles. *Am J Reprod Immunol* 2021;**85**:e13367.
59. Kalarikkal SP, Prasad D, Kasiappan R, Chaudhari SR, Sundaram GM. A cost-effective polyethylene glycol-based method for the isolation of functional edible nanoparticles from ginger rhizomes. *Sci Rep* 2020;**10**:4456.
60. Yin LF, Yan L, Yu Q, Wang J, Liu CH, Wang L, et al. Characterization of the microRNA profile of ginger exosome-like nanoparticles and their anti-inflammatory effects in intestinal caco-2 cells. *J Agric Food Chem* 2022;**70**:4725−34.
61. Umezu T, Takanashi M, Murakami Y, Ohno SI, Kanekura K, Sudo K, et al. Acerola exosome-like nanovesicles to systemically deliver nucleic acid medicine *via* oral administration. *Mol Ther Methods Clin Dev* 2021;**21**:199−208.
62. Savcı Y, Kırbaş OK, Bozkurt BT, Abdik EA, Taşlı PN, Şahin F, et al. Grapefruit-derived extracellular vesicles as a promising cell-free therapeutic tool for wound healing. *Food Funct* 2021;**12**:5144−56.
63. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol* 2006 [Chapter 3]:Unit 3.22.
64. Tauro BJ, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, et al. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. *Methods* 2012;**56**:293−304.
65. Zhang JJ, Nguyen LTH, Hickey R, Walters N, Wang XY, Kwak KJ, et al. Immunomagnetic sequential ultrafiltration (iSUF) platform for enrichment and purification of extracellular vesicles from biofluids. *Sci Rep* 2021;**11**:8034.
66. Sundaram PM, Casadei L, Lopez G, Braggio D, Balakirsky G, Pollock R, et al. Multi-layer micro-nanofluidic device for isolation and capture of extracellular vesicles derived from liposarcoma cell conditioned media. *J Microelectromech Syst* 2020;**29**:776−82.
67. Jia L, Li B, Fang C, Liang XY, Xie YJ, Sun XF, et al. Extracellular vesicles of mesenchymal stem cells are more effectively accessed through polyethylene glycol-based precipitation than by ultracentrifugation. *Stem Cells Int* 2022;**2022**:3577015.
68. Muraoka S, Jedrychowski MP, Yanamandra K, Ikezu S, Gygi SP, Ikezu T. Proteomic profiling of extracellular vesicles derived from cerebrospinal fluid of Alzheimer's disease patients: a pilot study. *Cells* 2020;**9**:1959.
69. Gondim BLC, Oshiro-Júnior JA, Fernanandes FHA, Nóbrega FP, Castellano LRC, Medeiros ACD. Plant extracts loaded in nanostructured drug delivery systems for treating parasitic and antimicrobial diseases. *Curr Pharm Des* 2019;**25**:1604−15.
70. Farley JT, Eldahshoury MK, de Marcos Lousa C. Unconventional secretion of plant extracellular vesicles and their benefits to human health: a mini review. *Front Cell Dev Biol* 2022;**10**:883841.
71. Dong L, Zieren RC, Horie K, Kim CJ, Mallick E, Jing Y, et al. Comprehensive evaluation of methods for small extracellular vesicles separation from human plasma, urine and cell culture medium. *J Extracell Vesicles* 2020;**10**:e12044.
72. Şahin F, Koçak P, Güneş MY, Özkan İ, Yıldırım E, Kala EY. *In vitro* wound healing activity of wheat-derived nanovesicles. *Appl Biochem Biotechnol* 2019;**188**:381−94.
73. Yang M, Liu XY, Luo QQ, Xu LL, Chen FX. An efficient method to isolate lemon derived extracellular vesicles for gastric cancer therapy. *J Nanobiotechnol* 2020;**18**:100.

74. Zhang Y, Bi JY, Huang JY, Tang YN, Du SY, Li PY. Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. *Int J Nanomed* 2020;**15**:6917−34.
75. Munir J, Lee M, Ryu S. Exosomes in food: health benefits and clinical relevance in diseases. *Adv Nutr* 2020;**11**:687−96.
76. Gelibter S, Marostica G, Mandelli A, Siciliani S, Podini P, Finardi A, et al. The impact of storage on extracellular vesicles: a systematic study. *J Extracell Vesicles* 2022;**11**:e12162.
77. Wang QL, Zhuang XY, Mu JY, Deng ZB, Jiang H, Zhang LF, et al. Delivery of therapeutic agents by nanoparticles made of grapefruit-derived lipids. *Nat Commun* 2013;**4**:1867.
78. Yang CH, Zhang MZ, Merlin D. Advances in plant-derived edible nanoparticle-based lipid nano-drug delivery systems as therapeutic nanomedicines. *J Mater Chem B* 2018;**6**:1312−21.
79. Logozzi M, Di Raimo R, Mizzoni D, Fais S. Nanovesicles from organic agriculture-derived fruits and vegetables: characterization and functional antioxidant content. *Int J Mol Sci* 2021;**22**:8170.
80. Mu JY, Zhuang XY, Wang QL, Jiang H, Deng ZB, Wang BM, et al. Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. *Mol Nutr Food Res* 2014;**58**:1561−73.
81. Zhang L, He FJ, Gao LN, Cong MH, Sun J, Xu JL, et al. Engineering exosome-like nanovesicles derived from *Asparagus cochinchinensis* can inhibit the proliferation of hepatocellular carcinoma cells with better safety profile. *Int J Nanomed* 2021;**16**:1575−86.
82. Deng ZB, Rong Y, Teng Y, Mu JY, Zhuang XY, Tseng M, et al. Broccoli-derived nanoparticle inhibits mouse colitis by activating dendritic cell AMP-activated protein kinase. *Mol Ther* 2017;**25**:1641−54.
83. Alfieri M, Leone A, Ambrosone A. Plant-derived nano and microvesicles for human health and therapeutic potential in nanomedicine. *Pharmaceutics* 2021;**13**:498.
84. Ha D, Yang NN, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. *Acta Pharm Sin B* 2016;**6**:287−96.
85. Kumar A, Sundaram K, Mu JY, Dryden GW, Sriwastva MK, Lei C, et al. High-fat diet-induced upregulation of exosomal phosphatidylcholine contributes to insulin resistance. *Nat Commun* 2021;**12**:213.
86. Henrich SE, McMahon KM, Plebanek MP, Calvert AE, Feliciano TJ, Parrish S, et al. Prostate cancer extracellular vesicles mediate intercellular communication with bone marrow cells and promote metastasis in a cholesterol-dependent manner. *J Extracell Vesicles* 2020;**10**:e12042.
87. Cao M, Yan HJ, Han X, Weng L, Wei Q, Sun XY, et al. Ginseng-derived nanoparticles alter macrophage polarization to inhibit melanoma growth. *J Immunother Cancer* 2019;**7**:326.
88. Wang XF, Chung KP, Lin WL, Jiang LW. Protein secretion in plants: conventional and unconventional pathways and new techniques. *J Exp Bot* 2017;**69**:21−37.
89. Zhang M, Liu L, Lin XB, Wang Y, Li Y, Guo Q, et al. A translocation pathway for vesicle-mediated unconventional protein secretion. *Cell* 2020;**181**:637−52.
90. Zhang MZ, Viennois E, Prasad M, Zhang YC, Wang LX, Zhang Z, et al. Edible ginger-derived nanoparticles: a novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. *Biomaterials* 2016;**101**:321−40.
91. Pocsfalvi G, Turiák L, Ambrosone A, Del Gaudio P, Puska G, Fiume I, et al. Protein biocargo of citrus fruit-derived vesicles reveals heterogeneous transport and extracellular vesicle populations. *J Plant Physiol* 2018;**229**:111−21.
92. Raimondo S, Naselli F, Fontana S, Monteleone F, Lo Dico A, Saieva L, et al. Citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress CML xenograft growth by inducing TRAIL-mediated cell death. *Oncotarget* 2015;**6**:19514−27.
93. Bezerra-Neto JP, de Araújo FC, Ferreira-Neto JRC, da Silva MD, Pandolfi V, Aburjaile FF, et al. Plant aquaporins: diversity, evolution and biotechnological applications. *Curr Protein Pept Sci* 2019;**20**:368−95.
94. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;**9**:654−9.
95. Rutter BD, Innes RW. Growing pains: addressing the pitfalls of plant extracellular vesicle research. *New Phytol* 2020;**228**:1505−10.
96. O'Brien K, Breyne K, Ughetto S, Laurent LC, Breakefield XO. RNA delivery by extracellular vesicles in mammalian cells and its applications. *Nat Rev Mol Cell Biol* 2020;**21**:585−606.
97. Yu B, Yang ZY, Li JJ, Minakhina S, Yang MC, Padgett RW, et al. Methylation as a crucial step in plant microRNA biogenesis. *Science* 2005;**307**:932−5.
98. Chen XY, Liu BL, Li XZ, An TT, Zhou Y, Li G, et al. Identification of anti-inflammatory vesicle-like nanoparticles in honey. *J Extracell Vesicles* 2021;**10**:e12069.
99. Zhao YY, Mo BX, Chen XM. Mechanisms that impact microRNA stability in plants. *RNA Biol* 2012;**9**:1218−23.
100. Lukasik A, Zielenkiewicz P. Plant microRNAs-novel players in natural medicine? *Int J Mol Sci* 2016;**18**:9.
101. Jain KK. An overview of drug delivery systems. *Methods Mol Biol* 2020;**2059**:1−54.
102. Woith E, Guerriero G, Hausman JF, Renaut J, Leclercq CC, Weise C, et al. Plant extracellular vesicles and nanovesicles: focus on secondary metabolites, proteins and lipids with perspectives on their potential and sources. *Int J Mol Sci* 2021;**22**:3719.
103. Vasvári G, Kalmár J, Veres P, Vecsernyés M, Bácskay I, Fehér P, et al. Matrix systems for oral drug delivery: formulations and drug release. *Drug Discov Today Technol* 2018;**27**:71−80.
104. Reinholz J, Landfester K, Mailänder V. The challenges of oral drug delivery *via* nanocarriers. *Drug Deliv* 2018;**25**:1694−705.
105. Stotz H, Brotherton D, Inal J. Communication is key: extracellular vesicles as mediators of infection and defence during host-microbe interactions in animals and plants. *FEMS Microbiol Rev* 2022;**46**:fuab044.
106. Song HL, Canup BSB, Ngo VL, Denning TL, Garg P, Laroui H. Internalization of garlic-derived nanovesicles on liver cells is triggered by interaction with CD98. *ACS Omega* 2020;**5**:23118−28.
107. Iravani S, Varma RS. Plant pollen grains: a move towards green drug and vaccine delivery systems. *Nano-Micro Lett* 2021;**13**:128.
108. Yang L, Gu TW, Xu Y, Liu YD, Zhang YP, Jiang ZH, et al. Plant polysaccharides as novel biomaterials for microcapsule construction and therapeutics delivery. *Int J Pharm* 2022;**625**:122137.
109. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. *Nature* 2019;**575**:299−309.
110. Tyner JW, Haderk F, Kumaraswamy A, Baughn LB, Van Ness B, Liu S, et al. Understanding drug sensitivity and tackling resistance in cancer. *Cancer Res* 2022;**82**:1448−60.
111. Yang M, Luo QQ, Chen X, Chen FX. Bitter melon derived extracellular vesicles enhance the therapeutic effects and reduce the drug resistance of 5-fluorouracil on oral squamous cell carcinoma. *J Nanobiotechnol* 2021;**19**:259.
112. Shaikh IA, Brown I, Schofield AC, Wahle KW, Heys SD. Docosahexaenoic acid enhances the efficacy of docetaxel in prostate cancer cells by modulation of apoptosis: the role of genes associated with the NF-kappaB pathway. *Prostate* 2008;**68**:1635−46.
113. Panigrahy D, Gilligan MM, Serhan CN, Kashfi K. Resolution of inflammation: an organizing principle in biology and medicine. *Pharmacol Ther* 2021;**227**:107879.
114. Ito Y, Taniguchi K, Kuranaga Y, Eid N, Inomata Y, Lee SW, et al. Uptake of microRNAs from exosome-like nanovesicles of edible plant juice by rat enterocytes. *Int J Mol Sci* 2021;**22**:3749.
115. Chen XY, Zhou Y, Yu JJ. Exosome-like nanoparticles from ginger rhizomes inhibited NLRP3 inflammasome activation. *Mol Pharm* 2019;**16**:2690−9.

116. Cai Y, Zhang LX, Zhang YJ, Lu R. Plant-derived exosomes as a drug-delivery approach for the treatment of inflammatory bowel disease and colitis-associated cancer. *Pharmaceutics* 2022;**14**:822.
117. Teng Y, Ren Y, Sayed M, Hu X, Lei C, Kumar A, et al. Plant-derived exosomal microRNAs shape the gut microbiota. *Cell Host Microbe* 2018;**24**:637−52.
118. Lei C, Mu JY, Teng Y, He LQ, Xu FY, Zhang XC, et al. Lemon exosome-like nanoparticles-manipulated probiotics protect mice from *C. d iff* infection. *iScience* 2020;**23**:101571.
119. Sarwa KK, Das PJ, Mazumder B. A nanovesicle topical formulation of Bhut Jolokia (hottest capsicum): a potential anti-arthritic medicine. *Expert Opin Drug Deliv* 2014;**11**:661−76.
120. Schuh C, Aguayo S, Zavala G, Khoury M. Exosome-like vesicles in *Apis mellifera* bee pollen, honey and royal jelly contribute to their antibacterial and pro-regenerative activity. *J Exp Biol* 2019;**222**: jeb208702.
121. Canup BS, Song HL, Le Ngo V, Meng XX, Denning TL, Garg P, et al. CD98 siRNA-loaded nanoparticles decrease hepatic steatosis in mice. *Dig Liver Dis* 2017;**49**:188−96.
122. Zhang Y, Wang BY, Lu FF, Wang L, Ding YH, Kang XY. Plant-derived antioxidants incorporated into active packaging intended for vegetables and fatty animal products: a review. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2021;**38**:1237−48.
123. Perut F, Roncuzzi L, Avnet S, Massa A, Zini N, Sabbadini S, et al. Strawberry-derived exosome-like nanoparticles prevent oxidative stress in human mesenchymal stromal cells. *Biomolecules* 2021;**11**:87.
124. Atzrodt CL, Maknojia I, McCarthy RDP, Oldfield TM, Po J, Ta KTL, et al. A guide to COVID-19: a global pandemic caused by the novel coronavirus SARS-CoV-2. *FEBS J* 2020;**287**:3633−50.
125. Wang YC, Wang JF, Ma JQ, Zhou Y, Lu R. Focusing on future applications and current challenges of plant derived extracellular vesicles. *Pharmaceuticals (Basel)* 2022;**15**:708.
126. Kalarikkal SP, Sundaram GM. Edible plant-derived exosomal microRNAs: exploiting a cross-kingdom regulatory mechanism for targeting SARS-CoV-2. *Toxicol Appl Pharmacol* 2021;**414**: 115425.
127. Xiao J, Feng SY, Wang X, Long K, Luo Y, Wang YH, et al. Identification of exosome-like nanoparticle-derived microRNAs from 11 edible fruits and vegetables. *PeerJ* 2018;**6**:e5186.
128. Teng Y, Xu FY, Zhang XC, Mu JY, Sayed M, Hu X, et al. Plant-derived exosomal microRNAs inhibit lung inflammation induced by exosomes SARS-CoV-2 Nsp12. *Mol Ther* 2021;**29**:2424−40.
129. Bai R, Guo JN, Ye XY, Xie YY, Xie T. Oxidative stress: the core pathogenesis and mechanism of Alzheimer's disease. *Ageing Res Rev* 2022;**77**:101619.
130. Kim DK, Rhee WJ. Antioxidative effects of carrot-derived nanovesicles in cardiomyoblast and neuroblastoma cells. *Pharmaceutics* 2021;**13**:1203.
131. Han X, Wei Q, Lv Y, Weng L, Huang HY, Wei QY, et al. Ginseng-derived nanoparticles potentiate immune checkpoint antibody efficacy by reprogramming the cold tumor microenvironment. *Mol Ther* 2022;**30**:327−40.
132. Stanly C, Alfieri M, Ambrosone A, Leone A, Fiume I, Pocsfalvi G. Grapefruit-derived micro and nanovesicles show distinct metabolome profiles and anticancer activities in the A375 human melanoma cell line. *Cells* 2020;**9**:2722.
133. Teng Y, Mu JY, Hu X, Samykutty A, Zhuang XY, Deng ZB, et al. Grapefruit-derived nanovectors deliver miR-18a for treatment of liver metastasis of colon cancer by induction of M1 macrophages. *Oncotarget* 2016;**7**:25683−97.
134. Manca ML, Casula E, Marongiu F, Bacchetta G, Sarais G, Zaru M, et al. From waste to health: sustainable exploitation of grape pomace seed extract to manufacture antioxidant, regenerative and prebiotic nanovesicles within circular economy. *Sci Rep* 2020;**10**:14184.
135. Ju SW, Mu JY, Dokland T, Zhuang XY, Wang QL, Jiang H, et al. Grape exosome-like nanoparticles induce intestinal stem cells and protect mice from DSS-induced colitis. *Mol Ther* 2013;**21**: 1345−57.
136. Stanly C, Kim H, Antonucci G, Fiume I, Guescini M, Kim KP, et al. Crosstalk between the immune system and plant-derived nanovesicles: a study of allergen transporting. *Front Bioeng Biotechnol* 2021;**9**:760730.
137. Lei C, Teng Y, He LQ, Sayed M, Mu JY, Xu FY, et al. Lemon exosome-like nanoparticles enhance stress survival of gut bacteria by RNase P-mediated specific tRNA decay. *iScience* 2021;**24**:102511.
138. Bruno SP, Paolini A, D'Oria V, Sarra A, Sennato S, Bordi F, et al. Extracellular vesicles derived from citrus sinensis modulate inflammatory genes and tight junctions in a human model of intestinal epithelium. *Front Nutr* 2021;**8**:778998.
139. Berger E, Colosetti P, Jalabert A, Meugnier E, Wiklander OPB, Jouhet J, et al. Use of nanovesicles from orange juice to reverse diet-induced gut modifications in diet-induced obese mice. *Mol Ther Methods Clin Dev* 2020;**18**:880−92.
140. Li ZF, Wang HZ, Yin HR, Bennett C, Zhang HG, Guo PX. Arrowtail RNA for ligand display on ginger exosome-like nanovesicles to systemic deliver siRNA for cancer suppression. *Sci Rep* 2018;**8**: 14644.
141. Ilhan-Ayisigi E, Ulucan F, Saygili E, Saglam-Metiner P, Gulce-Iz S, Yesil-Celiktas O. Nano-vesicular formulation of propolis and cytotoxic effects in a 3D spheroid model of lung cancer. *J Sci Food Agric* 2020;**100**:3525−35.
142. Sriwastva MK, Deng ZB, Wang B, Teng Y, Kumar A, Sundaram K, et al. Exosome-like nanoparticles from mulberry bark prevent DSS-induced colitis *via* the AhR/COPS8 pathway. *EMBO Rep* 2022;**23**: e53365.
143. Haque MN, Mohibbullah M, Hong YK, Moon IS. Calotropis gigantea promotes neuritogenesis and synaptogenesis through activation of NGF-TrkA-Erk1/2 signaling in rat hippocampal neurons. *Am J Chin Med* 2018;**46**:1861−77.
144. Urzì O, Gasparro R, Ganji NR, Alessandro R, Raimondo S. Plant-RNA in extracellular vesicles: the secret of cross-kingdom communication. *Membranes (Basel)* 2022;**12**:352.
145. Urzì O, Raimondo S, Alessandro R. Extracellular vesicles from plants: current knowledge and open questions. *Int J Mol Sci* 2021;**22**: 5366.
146. Zhang MZ, Xiao B, Wang H, Han MK, Zhang Z, Viennois E, et al. Edible ginger-derived nano-lipids loaded with doxorubicin as a novel drug-delivery approach for colon cancer therapy. *Mol Ther* 2016;**24**: 1783−96.
147. Kim J, Li SY, Zhang SY, Wang JX. Plant-derived exosome-like nanoparticles and their therapeutic activities. *Asian J Pharm Sci* 2022;**17**:53−69.
148. Mao YL, Han MQ, Chen CS, Wang XD, Han JN, Gao YK, et al. A biomimetic nanocomposite made of a ginger-derived exosome and an inorganic framework for high-performance delivery of oral antibodies. *Nanoscale* 2021;**13**:20157−69.
149. Wang QL, Ren Y, Mu JY, Egilmez NK, Zhuang XY, Deng ZB, et al. Grapefruit-derived nanovectors use an activated leukocyte trafficking pathway to deliver therapeutic agents to inflammatory tumor sites. *Cancer Res* 2015;**75**:2520−9.
150. Wang BM, Zhuang XY, Deng ZB, Jiang H, Mu JY, Wang QL, et al. Targeted drug delivery to intestinal macrophages by bioactive nanovesicles released from grapefruit. *Mol Ther* 2014;**22**:522−34.
151. Chen NX, Sun JF, Zhu ZH, Cribbs AP, Xiao B. Edible plant-derived nanotherapeutics and nanocarriers: recent progress and future directions. *Expert Opin Drug Deliv* 2022;**19**:409−19.
152. Assas MB, Levison SE, Little M, England H, Battrick L, Bagnall J, et al. Anti-inflammatory effects of infliximab in mice are independent of tumor necrosis factor α neutralization. *Clin Exp Immunol* 2017;**187**:225−33.
153. Cai Q, He BY, Kogel KH, Jin HL. Cross-kingdom RNA trafficking and environmental RNAi-nature's blueprint for modern crop protection strategies. *Curr Opin Microbiol* 2018;**46**: 58−64.
154. Li D, Yao XL, Yue JX, Fang YP, Cao GF, Midgley AC, et al. Advances in bioactivity of microRNAs of plant-derived exosome-like

nanoparticles and milk-derived extracellular vesicles. *J Agric Food Chem* 2022;**70**:6285−99.
155. Zhang MZ, Wang XY, Han MK, Collins JF, Merlin D. Oral administration of ginger-derived nanolipids loaded with siRNA as a novel approach for efficient siRNA drug delivery to treat ulcerative colitis. *Nanomedicine (Lond)* 2017;**12**:1927−43.
156. Large DE, Abdelmessih RG, Fink EA, Auguste DT. Liposome composition in drug delivery design, synthesis, characterization, and clinical application. *Adv Drug Deliv Rev* 2021;**176**:113851.
157. Sarkar S, Levi-Polyachenko N. Conjugated polymer nano-systems for hyperthermia, imaging and drug delivery. *Adv Drug Deliv Rev* 2020;**163**−**164**:40−64.
158. Ghezzi M, Pescina S, Padula C, Santi P, Del Favero E, Cantù L, et al. Polymeric micelles in drug delivery: an insight of the techniques for their characterization and assessment in biorelevant conditions. *J Control Release* 2021;**332**:312−36.
159. Kumar N, Chamoli P, Misra M, Manoj MK, Sharma A. Advanced metal and carbon nanostructures for medical, drug delivery and bioimaging applications. *Nanoscale* 2022;**14**:3987−4017.
160. Chen YS, Lin EY, Chiou TW, Harn HJ. Exosomes in clinical trial and their production in compliance with good manufacturing practice. *Ci Ji Yi Xue Za Zhi* 2019;**32**:113−20.
161. Wu K, Xing F, Wu SY, Watabe K. Extracellular vesicles as emerging targets in cancer: recent development from bench to bedside. *Biochim Biophys Acta Rev Cancer* 2017;**1868**:538−63.
162. Di Gioia S, Hossain MN, Conese M. Biological properties and therapeutic effects of plant-derived nanovesicles. *Open Med* 2020;**15**:1096−122.
163. Sarvarian P, Samadi P, Gholipour E, Shams Asenjan K, Hojjat-Farsangi M, Motavalli R, et al. Application of emerging plant-derived nanoparticles as a novel approach for nano-drug delivery systems. *Immunol Invest* 2022;**51**:1039−59.
164. Logozzi M, Di Raimo R, Mizzoni D, Fais S. The potentiality of plant-derived nanovesicles in human health − a comparison with human exosomes and artificial nanoparticles. *Int J Mol Sci* 2022;**23**:4919.
165. Karamanidou T, Tsouknidas A. Plant-derived extracellular vesicles as therapeutic nanocarriers. *Int J Mol Sci* 2021;**23**:191.
166. Shariare MH, Noor HB, Khan JH, Uddin J, Ahamad SR, Altamimi MA, et al. Liposomal drug delivery of Corchorus olitorius leaf extract containing phytol using design of experiment (DoE): *in-vitro* anticancer and *in-vivo* anti-inflammatory studies. *Colloids Surf B Biointerfaces* 2021;**199**:111543.
167. Cong MH, Tan SY, Li SM, Gao LN, Huang LQ, Zhang HG, et al. Technology insight: plant-derived vesicles-how far from the clinical biotherapeutics and therapeutic drug carriers?. *Adv Drug Deliv Rev* 2022;**182**:114108.
168. Rome S. Biological properties of plant-derived extracellular vesicles. *Food Funct* 2019;**10**:529−38.
169. Bosch S, de Beaurepaire L, Allard M, Mosser M, Heichette C, Chrétien D, et al. Trehalose prevents aggregation of exosomes and cryodamage. *Sci Rep* 2016;**6**:36162.
170. Chernyshev VS, Rachamadugu R, Tseng YH, Belnap DM, Jia Y, Branch KJ, et al. Size and shape characterization of hydrated and desiccated exosomes. *Anal Bioanal Chem* 2015;**407**:3285−301.
171. Yu LL, Deng Z, Liu L, Zhang WB, Wang CX. Plant-derived nanovesicles: a novel form of nanomedicine. *Front Bioeng Biotechnol* 2020;**8**:584391.